Diclofenac for Acute Pain in Children: Pharmacokinetics and Safety

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PhD Thesis

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Plagiarism Statement

This thesis describes research conducted in the School of Pharmacy, University of London between February 2004 and August 2007 under the supervision of Professor Ian Wong and Dr Imogen Savage. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

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Signature Date

To: Victor Standing

"*Ma guai a chi cede alla tentazione di scambiare una ipotesi elegante con una certezza.*"

Primo Levi, Il Sistema Periodico, 1975.

"But there is trouble for those who surrender to the temptation of mistaking an elegant hypothesis for a certainty."

Primo Levi, The Periodic Table, 1975.

Abstract

Diclofenac is commonly used 'off-label' for acute pain in children, and it has been shown to be effective for this indication. There is a five-fold range (0.5 to 2.5mg/kg) in dosing of diclofenac for acute pain in paediatric clinical studies, and little published safety information is available. The metabolism of diclofenac to 4'-hydroxydiclofenac is mediated by CYP2C9, the expression of which may differ during development.

Three studies have been undertaken to answer the questions: What dose of diclofenac should be given to children with acute pain? What are the adverse effects of diclofenac in children treated for acute pain? Does the expression of CYP2C9 change with age in children aged one to 12 years? The three studies carried out were: A population pharmacokinetic study on a paediatric day surgery ward investigating a new diclofenac oral suspension, results pooled with adult data supplied by the manufacturer and analysed with NONMEM to produce dosing guidelines; a clinical safety study to ascertain common adverse reactions of diclofenac in children with acute pain, followed by a systematic literature review to investigate the type and incidence of rare adverse effects; and an investigation of the influence of age and CYP2C9 genotype on the formation of 4'-hydroxydiclofenac in children aged one to 12 years using data collected during the pharmacokinetic study.

The optimum dose of diclofenac for acute pain in children is 1mg/kg. Diclofenac appeared to cause similar types of adverse reactions in children and adults, although the incidence of gastrointestinal bleeding is possibly lower in children. When diclofenac is used as part of the analgesic regimen in the peri-operative period, children suffer less nausea and vomiting, and no increase in operative bleeding. No differences were found in the expression of CYP2C9 estimated using diclofenac 4'-hydroxylation in children aged one to 12 years, which would appear to confirm *in vitro* findings in paediatric liver samples.

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The final and largest acknowledgement is reserved for the patients who took part, without whom the clinical studies would not have been possible.

Publications

To-date, the results of this thesis have been presented on the following occasions:

Published Protocol:

Standing JF, Pritchard D, Savage I, Waddington M. Diclofenac for acute pain in children. [Protocol] Cochrane Database of Systematic Reviews:CD005538.

Oral presentations:

Standing JF, Howard RF, Johnston A, Savage I, Wong ICK. Developmental pharmacokinetics of diclofenac for acute pain. PAGE Meeting, Copenhagen, June 2007. Abstract and slides available at: http://www.page-meeting.org/default.asp?abstract=1086

Standing JF, Howard RF, Keady S, Ooi K, Savage I, Wong ICK. Safety of diclofenac for acute pain in children. NPPG Conference, November 2006.

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Poster presentations:

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Standing JF, Howard RF, Johnston A, Savage I, Wong ICK. Pharmacokinetics of diclofenac suspension in adults. London School of Pharmacy, PhD Research Day, April 2006.

The following publications in the field of paediatric medicines were undertaken during the course of this thesis, although they do not directly relate to it:

Standing JF, Khaki ZF, Wong ICK. 2005. Poor formulation information in published paediatric drug trials. Pediatrics, 116:E559-62.

Standing JF, Tuleu C. 2005. Paediatric formulations – getting to the heart of the problem. International Journal of Pharmaceutics, 300:56-66.

Standing JF. 2006. Long-term bosentan treatment in children with pulmonary arterial hypertension. Journal of the American College of Cardiology. [Letter] 47, 1914-5.

Standing JF, Wong ICK. 2004. Chlorproguanil-dapsone for malaria. [Letter] Lancet, 364:1752-53.

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Chapter ONE: Literature Review

1.1 Diclofenac

1.1.1 Diclofenac History

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used to treat both acute and chronic pain, and inflammation (*Martindale*, 2007). The first of this class of compounds was salicylic acid, traditionally extracted from willow bark but a constituent of several plants which have been used as medicines since ancient Egyptian times (Vane JR & Botting RM, 1998). Salicylic acid was first chemically synthesised in 1860, the first synthetic NSAID produced after this was phenylbutazone in 1952, which was followed by drugs such as mefanamic acid, indomethacin and ibuprofen. Diclofenac was first marketed in 1974 and was discovered by medicinal chemists at Ciba-Geigy as the result of structure-activity related design based on the NSAIDs commonly available at the time: phenylbutazone, mefanamic acid and indomethacin; all are weak acids, have similar lipophilicity and contain two aromatic rings twisted in relation to each other (Sallmann AR, 1986). The investigators rightly hypothesised that these features were important for anti-inflammatory activity, and screening of several such compounds yielded the NSAID now known as diclofenac.

1.1.2 Structure and Chemistry

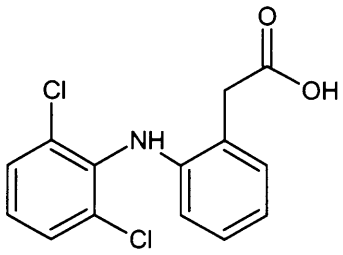


Figure 1.1 Diclofenac structure.

Diclofenac {[2-(2,6-dichlorophenyl)amino]phenylacetic acid}(figure 1.1) has a pKa in water of between 3.8 and 4.2. At low pH diclofenac is poorly soluble in water; solubility rapidly increases at pH values above the pKa, and dissolution is complete within two

minutes at pH 7.5. The partition coefficient measured by the Log of partition (Log P) between octanol and water at 25°C is 4.0 for unionised diclofenac (*Codex*, 1993), and the distribution coefficient at physiological pH (Log $D_{pH7.4}$) is 1.1 (Onoue S et al, 2007).

1.1.3 Pharmacology

The NSAIDs are a structurally diverse group of compounds with similar therapeutic and adverse drug reactions, suggesting a common mode of action. It is now widely understood that this common mode of action is primarily through the inhibition of prostaglandin synthesis.

Mechanical stress, or the presence of cytokines or growth factors, liberates arachidonic acid stored in the membrane phospholipids of most cells (Cashman J & McNaulty G, 1995). Arachidonic acid is a 20-carbon fatty acid released from the cell membrane by the action of phospholipase, which is liberated in response to substance P released by tissue damage (Ochroch EA et al, 2003). Cyclo-oxygenase (COX), also known as prostaglandin endoperoxide synthase, catalyses the conversion of arachidonic acid to prostaglandin H2 through sequential cyclisation and oxidation (Garavito RM & DeWitt DL, 1999). The action of specific synthases then forms prostacyclin, thromboxanes and other prostaglandins important in the inflammatory response (Burian M & Geisslinger G, 2005).

Inflammatory pain is characterised by an increased response (sensitisation) to noxious stimuli (hyperalgesia) or a sensitisation to previously non-noxious stimuli (allodynia) (Burian M & Geisslinger G, 2005). Pain is signalled by nociceptors either through transduction along unmyelinated C fibres, or conduction via Aδ primary sensory fibres. Synaptic transfer involving cells of the dorsal horn, which project into the cortex via a relay in the thalamus, forms the transmission phase of pain perception (Burian M & Geisslinger G, 2005). Sensitisation involved in inflammatory pain is mediated by prostanoids formed from arachidonic acid catalysed by COX. Whilst they have no direct effect on nociceptors, these prostanoids cause nociceptor sensitisation to chemical mediators such as bradykinin and serotonin (Cashman J & McNaulty G, 1995, Dray A, 1995).

Diclofenac probably inhibits COX reversibly through hydrogen bonding at arginine-120, thereby competing with arachidonic acid (Garavito RM & DeWitt DL, 1999). Two main isoforms of COX have been isolated, COX-1 and COX-2 (Vane JR & Botting RM, 1998), which are structurally and functionally very similar membrane-bound proteins found in the endoplasmic reticulum and nuclear envelope. The existence of a COX-3 in humans remains controversial although inhibition of this could be a possible mode of action of paracetamol (Botting RM, 2000). Diclofenac inhibits both COX-1 and COX-2 and has recently been shown to be 20 times more potent in inhibiting COX-2 over COX-1 (Cryer B & Feldman M, 1998, Capone ML et al, 2007). Unlike ibuprofen and naproxen, which competitively bind at the COX-2 active site, diclofenac displays time-dependent inhibition even at high concentrations of arachidonic acid (Blobaum AL & Marnett LJ, 2007, Rowlinson SW et al, 2003).

COX-1 is detectable in most cell types and is thought to undertake many homeostatic 'house-keeping' roles. These include the formation of prostaglandins with gastrointestinal cytoprotective effects through decreasing gastric secretions, increasing mucosal blood flow and increasing the production of gastric mucus. COX-1 is responsible for producing vasodilatory prostaglandins regulating renal blood flow (Vane JR & Botting RM, 1998) and its presence in several kidney cell-types has suggested a role for COX-1 in renal water balance (Morita I, 2002).

It was thought that COX-1 was non-inducible (Vane JR & Botting RM, 1998) but it has more recently been found to be up-regulated in conditions such as shear stress on endothelial cells (Morita I, 2002). By contrast, in most cells COX-2 is undetectable with expression increasing in pathological conditions such as sites of inflammation and some cancers (Burian M & Geisslinger G, 2005). This marked up-regulation at inflammation sites prompted the theory that COX-2 inhibition is largely responsible for the therapeutic effects of NSAIDs, whilst inhibition of COX-1's 'house-keeping' functions may cause adverse drug reactions. This theory led to the development of specific COX-2 inhibitors, which should have the therapeutic benefits of NSAIDs whilst avoiding their adverse drug reactions (Vane JR & Botting RM, 1998). Support for this is illustrated by the example of platelet aggregation mediated by thromboxane A2. Platelets only express COX-1, which

converts arachidonic acid to thromboxane A2 in response to agonists such as thrombin. Aspirin irreversibly inhibits COX-1 by selective acetylation of serine at the 529 position (Simmons DL et al, 2004). Whilst aspirin causes almost complete thromboxane-mediated inhibition of platelet aggregation, this is not seen with selective COX-2 inhibitors (Capone ML et al, 2007), and it is also the case that COX-1 deficient mice have reduced platelet aggregation (Morita I, 2002). Prolonged COX-1 inhibition in the gastric mucosa is a compelling explanation for gastro-duodenal lesions seen in patients taking diclofenac (Catalano MA, 1986). COX-1 deficient mice are resistant to indomethacin-induced gastric ulceration, although interestingly COX-1 deficiency did not cause gastric pathology in itself (Morita I, 2002), suggesting the possibility of compensatory processes maintaining gastro-intestinal function in long-term COX-1 absence or inhibition.

As COX-2 expression is up-regulated at sites of inflammation (Morita I, 2002), it is tempting to expect that diclofenac has primarily peripheral activity. However, despite achieving similar tissue concentrations measured by microdialysis, oral systemic diclofenac was found to be more effective than topical diclofenac in a randomised placebo controlled study on experimentally induced mechanical pain. This suggests that diclofenac has a central mode of action possibly accounting for as much as 40 percent of its analgesic activity (Burian M et al, 2003). The central nervous system (CNS) is one of the few sites where COX-2 is constitutively expressed, and it is also susceptible to further up-regulation in response to peripheral inflammation. COX-1 has also been shown to have involvement in spinal nociceptive processing, becoming up-regulated after nerve injury and post-operatively (Burian M & Geisslinger G, 2005). This provides a plausible central mechanism for analgesia induced by diclofenac, suggesting central COX-2 has involvement in inflammatory pain, and COX-1 in the CNS could be a useful target for operative and neuropathic pain.

Whilst COX inhibition is a possible mechanism for the peripheral and central analgesic effects of diclofenac, several other pathways have been suggested involving direct action on nociceptors. Diclofenac has been found to down-regulate noiciceptor stimulation by activating the arginine-nitiric oxide cyclic guanosine monophosphate (GMP) system (Tonussi CR & Ferreira SH, 1994) and it may also have analgesic effects by blocking

presynaptic α-1 adrenergic receptors thereby decreasing transmitter release and therefore pain transmission (Pinardi G et al, 2002). Although the mechanism is unclear, diclofenac seems to activate outward K^+ current channels in rat cerebellar granule cells, which could potentially decrease neuronal excitability and therefore painful stimuli (Liu LY et al, 2005). Diclofenac may also interact with sodium ion channels as it has been found to inhibit small nociceptive neurones in the dorsal root ganglion (Lee HM et al, 2003) although both of these studies used higher concentrations of diclofenac than would be expected with therapeutic dosing. A reduction in pain induced by acidic pH by inhibiting the expression of acid-sensitising ion channels in sensory neurones may also occur with diclofenac (Voilley N et al, 2001).

It is clear that the NSAIDs including diclofenac seem to act on several pharmacological targets although the therapeutic effects are mainly mediated by COX-2 inhibition, whilst some of the adverse drug reactions are due to COX-1 inhibition. However, as COX-1 inhibition may contribute some central analgesic activity, recent findings of cardiac mortality associated with prolonged use of selective COX-2 inhibitors (Juni P et al, 2004), and the possible existence of COX-3 (Simmons DL et al, 2004), all suggest that the delineation of therapeutic effects and adverse drug reactions between isoforms is not straightforward.

1.1.4 Pharmacokinetics

1.1.4.1 Absorption

Diclofenac is usually administered orally, although it can be given rectally, topically, or parenterally by either intravenous or intramuscular injection (*Martindale*, 2007). Bioavailability of unchanged drug following dosing with enteric-coated tablets is approximately 54 percent (Willis JV et al, 1979), with total drug absorption reaching approximately 90 percent (Davies NM & Anderson KE, 1997).

In fasted individuals, after administration of a diclofenac-containing buffered aqueous solution, absorption is rapid with maximum concentration time (t_{max}) attained within ten minutes, and a single peak is seen in the plasma concentration versus time curve (Degen PH et al, 1988, Lau HSH et al, 1989, Mahmood I, 1996). Mean maximum concentrations

(C_{max}) and area under the plasma concentration versus time curve (AUC) increased proportionally with single doses of 50mg, 100mg and 150mg, and t_{max} and elimination half-life ($t_{1/2}$) were similar, suggesting there is linear absorption and elimination over this dose range (Lau HSH et al, 1989).

Dosing with a buffered aqueous solution of diclofenac 15 minutes after a light snack can delay t_{max} by up to two hours and the phenomenon of multiple peaks is seen in the plasma concentration versus time curve (Chan KKH et al, 1990). When dispersible tablets of diclofenac are given to fasted individuals, absorption is rapid but double and sometimes multiple peaks are also observed (Lotsch J et al, 2000, Macia MA et al, 1995). Slower absorption rates are seen with sustained release tablets although double/multiple peaks can frequently occur (Chan KKH et al, 1990, Idkaidek NM et al, 1998, Mahmood I, 1996) whereas enteric-coated tablets tend to give a single peak with marked variation in inter-individual absorption lag times (Chan KKH et al, 1990, Idkaidek NM et al, 1998, Lotsch J et al, 2000, Willis JV et al, 1979), the variability in lag time being further increased when tablets are taken with food (Willis JV et al, 1983).

The observation that some patients exhibit single peaks and some show double or multiple peaks in the absorption profile when given the same formulation and same state of nourishment (usually fasted) has three possible explanations. The first is enterohepatic circulation whereby unchanged diclofenac (or diclofenac conjugates excreted in the bile which then liberate diclofenac in the gut) allow the drug to be reabsorbed causing a second peak. Although this is a theoretical possibility as diclofenac does undergo enterohepatic recirculation in rats (Whittle BJR, 2004), only approximately 30 percent of a dose is excreted in the bile in man (Davies NM & Anderson KE. 1997) making a large second peak unlikely. Furthermore, pharmacokinetic profiles of patients given intravenous or enteric-coated diclofenac do not show double/multiple peaks (Chan KKH et al, 1990, Idkaidek NM et al, 1998, Korpela R & Olkkola KT, 1990, Lotsch J et al, 2000, Willis JV et al, 1979), suggesting that enterohepatic recirculation alone is not significant enough to cause noticeable changes in the pharmacokinetic profile. It would therefore seem that double, or rarely multiple peaks are related to the absorption process at the point where diclofenac leaves the stomach.

The second possible cause of double/multiple peaks is the pH dependent dissolution of diclofenac. At low pH diclofenac is un-ionised and virtually insoluble in aqueous media (*Codex*, 1993), meaning it cannot be absorbed. It is therefore possible that pH dependent absorption windows exist for (non-buffered) diclofenac solutions in the upper part of the small intestine, causing the drug to enter and leave solution. This would explain the double peaks in soluble tablet profiles and also why the absorption profile, potentially caused by dissolution-rate changes, of slow release tablets is variable.

The final possible explanation is that part of the dose remains in the stomach when given as a soluble tablet. In general, when a liquid enters the fasted stomach it will pass directly through to the duodenum (Washington N et al, 2002). However, it is possible that a proportion of a soluble tablet, either because it contains large particles prompting contraction of the pyloric sphincter, or due to turbulence caused by the slight upward curvature of the distal antrum (Washington N et al, 2002), remains in the stomach. This portion will not be absorbed as the low pH means that most diclofenac will not be in solution, and the small fraction that is will be negligibly absorbed due to the relatively low mucosal surface area of the stomach. The second peak will occur when the remaining portion of the dose is released into the small intestine. This is a less likely explanation than the pH dependent absorption window theory as it cannot explain double/multiple peaks seen with slow release tablets or the single peak seen with buffered solutions given to fasted individuals. It may explain the multiple peaks seen with a buffered solution given to subjects who have recently eaten, but this could be due to increased volume of stomach contents causing decreased buffering capacity, thereby lowering the pH and causing diclofenac to precipitate.

Theoretically it is most likely that pH dependent dissolution causes the multiple peak absorption profile often seen with diclofenac. Supporting evidence for this theory can be found in a study where human subjects swallowed enteric-coated and slow-release diclofenac tablets attached to a radiotelemetric pH monitor, with absorption measured by plasma concentration and temporal correlation made with pH (Chan KKH et al, 1990). A lag time of up to one and a half hours occurred between gastric emptying and the start of

absorption, suggesting that the pH and/or fluid volume in the proximal part of the small intestine may be insufficient to dissolve the enteric coating or allow diclofenac to dissolve. Despite the differences in absorption profiles, bioequivalence in terms of AUC comparison is frequently achieved with various oral formulations (Chan KKH et al, 1990, Idkaidek NM et al, 1998, Macia MA et al, 1995, Maggi CA et al, 1990), although rectal bioavailability may be slightly lower (Idkaidek NM et al, 1998).

1.1.4.2 Distribution

Over 99.7 percent of circulating diclofenac is bound to plasma proteins and volume of distribution (V_D) is reported to be 0.1-0.2 L/kg (Davies NM & Anderson KE, 1997). Whilst this suggests that most of the drug in the body remains in the circulation, diclofenac does distribute into the tissues with measurable concentrations found in the synovial fluid (Fowler PD et al, 1983) and cerebrospinal fluid (CSF) (Zecca L et al, 1991). Interestingly, higher concentrations of the principal metabolite 4'-hydroxydiclofenac are found in the CSF than diclofenac itself (Zecca L et al, 1991). Diclofenac is a relatively small molecule with low lipid solubility at physiological pH, allowing it to cross biological membranes through intercellular junctions. Diclofenac has been found to accumulate in inflamed tissue (Brune K et al, 1992), possibly due to its high affinity for protein binding.

1.1.4.3 Metabolism

Diclofenac is extensively metabolised, mainly by hydroxylation of the phenyl groups with or without conjugation. Some metabolites are formed around the carboxylic acid and amino group forming indolinone derivatives (Stierlin H et al, 1979a). At least 11 metabolites have been identified (Stierlin H et al, 1979a) with the major ones being 4'-hydroxydiclofenac, 5-hydroxydiclofenac and diclofenac/diclofenac phase I metabolite glucuronides (Stierlin H & Faigle JW, 1979b, Tang W, 2003). The main diclofenac metabolite structures are given in figure 1.2.

The formation of 5-hydroxydiclofenac is catalysed by cytochrome P450 (CYP) 3A4 (Shen S et al, 1999), whereas the formation of 4'-hydroxydiclofenac is catalysed predominantly by CYP2C9 (Leeman T et al, 1993, Tang W, 2003). These oxidative products may be further conjugated with glucuronide, which in addition to the glucuronidation of unchanged

diclofenac, are catalysed by UDP-glucuronosyltransferase (UGT) isoforms 1A3, 1A9 2B4 and 2B7 (Kuehl GE et al, 2005).

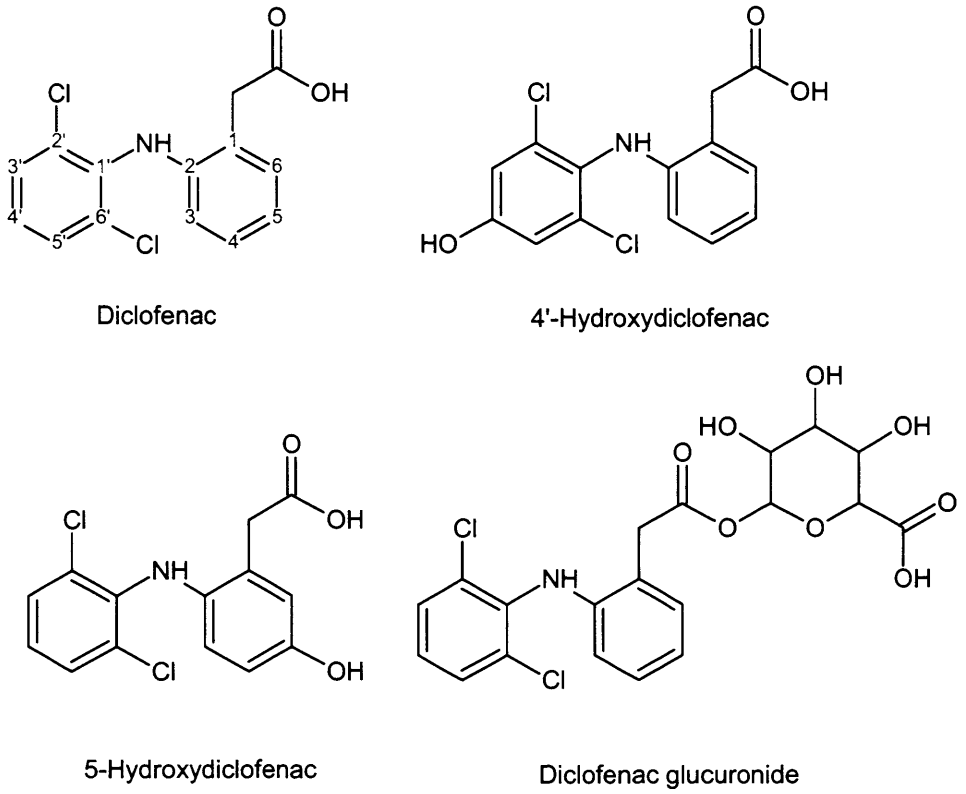


Figure 1.2: Structure of diclofenac and its major metabolites.

As the formation of 4'-hydroxydiclofenac is catalysed by the polymorphic enzyme CYP2C9, variants of which may have reduced catalytic activity (Miners JO & Birkett DJ, 1998), the ratio of diclofenac:4'-hydroxydiclofenac is a potential phenotypic indicator of CYP2C9 activity. The CYP2C9 gene is located on chromosome ten and is the largest contributor to drug metabolising capacity in the CYP2C subfamily (Rettie AE & Jones JP, 2005). The CYP2C9 protein consists of a 490 amino acid sequence coded by a 55kbase gene with nine exons (Xie HG et al, 2002). To date 30 single nucleotide polymorphisms

(SNPs) in the coding region of the CYP2C9 gene have been discovered (Maekawa K et al, 2006), some of which may affect protein function, and therefore diclofenac 4'-hydroxylation, through amino acid changes.

Several clinical studies have investigated whether there is a correlation in diclofenac:4'-hydroxydiclofenac ratio with different genotypes *in vivo* (Aithal GP et al, 2000, Dorado P et al 2003a, Dorado P et al 2003b, Kirchheiner J et al, 2002, Morin S et al, 2001, Shimamoto J et al, 2000, Yasar U et al, 2001). Mostly these studies have been underpowered in that they contain too few 'poor-metaboliser' genotypes (Dickinson GL et al, 2007). Some CYP2C9 pharmacogenomic studies with diclofenac used enteric-coated tablets and only collected urine for eight hours post-dose (absorption lag can be more than seven hours with enteric-coated diclofenac (Macia MA et al, 1995)). Furthermore, urinary recovery of 4'-hydroxydiclofenac was often undertaken to calculate the amount formed; the problem with this being that diclofenac glucuronide can be 4'-hydroxylated by CYP2C8 (Kumar S et al, 2002), and glucuronidated metabolites are readily hydrolysed (Davies NM & Anderson KE, 1997), potentially resulting in 4'-hydroxydiclofenac formed by CYP2C8 being liberated from the 4'-hydroxydiclofenac-glucuronide in urine. However, one group with the largest number of participants (102) has found decreased 4'-hydroxydiclofenac levels in the urine of CYP2C9 *1/*3 and *2/*3 patients (Dorado P et al 2003a, Dorado P et al 2003b), suggesting that there probably is some correlation between CYP2C9 genotype and 4'-hydroxydiclofenac formation.

The relative speed and ease with which genotyping for drug-metabolising enzymes can now be undertaken (Sistonen J et al, 2005) means that the use of probe drugs, such as diclofenac clearance to 4'-hydroxydiclofenac to determine genotype, is unnecessary. However, CYP2C9 expression or activity (phenotype) cannot so easily be determined, and this is an area where probe drugs could prove useful. Despite tolbutamide probably being the optimum marker of CYP2C9 activity (Fuhr U et al, 2007), some groups do use diclofenac as a CYP2C9 probe in drug cocktail interaction studies (Krosser S et al, 2006).

1.1.4.4 Elimination

Between 60 and 70 percent of a diclofenac dose is eliminated in the urine, the common forms being unchanged diclofenac (6.5 percent), 4'-hydroxydiclofenac (14.5 percent), 5-hydroxydiclofenac (5.5 percent) and small amounts of several other other hydroxylated metabolites; glucuronide conjugates of diclofenac and hydroxylated metabolites accounting for the remaining products (Degen PH et al, 1988, Stierlin H & Faigle JW, 1979b). Approximately 30 percent of a dose is excreted in the faeces (Davies NM & Anderson KE, 1997) with five percent being unchanged diclofenac, 40 percent as 4'-hydroxydiclofenac and 11 percent as 5-hydroxydiclofenac (Stierlin H & Faigle JW, 1979b).

Diclofenac and its hydroxy metabolites are rapidly cleared from the systemic circulation with terminal elimination $t_{1/2}$ of one to two hours, two to three hours and one to two-and-a-half hours for diclofenac, 4'-hydroxydiclofenac and 5-hydroxydiclofenac respectively (Degen PH et al, 1988, Landsdorp D et al, 1990). The only exception to the rapid elimination is 3'-hydroxy-4'-methoxydiclofenac which has a t_{max} of 12 hours and elimination $t_{1/2}$ of 80 hours. This metabolite has been found to accumulate on repeated dosing but appears not to have any pharmacological activity (Degen PH et al, 1988).

1.1.5 Pharmacodynamics

1.1.5.1 Beneficial effects

Diclofenac is commonly used in inflammatory conditions such as rheumatoid arthritis, oesteoarthritis, ankylosing spondylitis, bursitis and tendonitis (*Martindale*, 2007). The fact that diclofenac and 4'-hydroxydiclofenac levels in the synovial fluid of patients with rheumatoid arthritis were found to be higher than plasma levels between four and eight hours post-dose (Fowler PD et al, 1983), probably due to a lag in the distribution to peripheral areas, may mean anti-inflammatory action is delayed compared with plasma levels. Clinical studies conclude there does seem to be a dose-response relationship with NSAIDs in relation to anti-inflammatory activity, although a ceiling effect is reached at standard therapeutic dosing. This is highlighted by the anti-inflammatory action of diclofenac seeming to be equipotent to most other NSAIDs at usual therapeutic doses (Todd PA & Sorkin EM, 1988). However, diclofenac 100mg/day (usual dose 150mg/day) was found to have greater anti-inflammatory effects compared with a relatively low dose of

naproxen 500mg/day (usual dose 1250mg/day) (Todd PA & Sorkin EM, 1988), suggesting a degree of dose-response with the anti-inflammatory effect of NSAIDs.

The second major therapeutic use of diclofenac is for acute pain such as renal colic, migraine and post-operative pain (*Martindale*, 2007). In animal studies, the analgesic activity of diclofenac is at least 25-fold more potent than aspirin and three-fold more potent than ibuprofen (Todd PA & Sorkin EM, 1988). Diclofenac 100mg soluble tablets seemed to decrease experimentally induced pain when measured by visual analogue scale and electroencephalogram more when compared with a 50mg dose, but the difference was not statistically significant (Lotsch J et al, 2000). The linking of pharmacokinetic and pharmacodynamic data in this study may have been impaired by the relative crudeness of the pharmacokinetic model used; despite seeing double peaks after collecting rich pharmacokinetic data, which are to be expected with a diclofenac immediate release formulation, the authors chose a simple first-order absorption model with an associated lag time. This potential inadequacy of the pharmacokinetic model and the unexplained variability within may have affected the ability to link it with pharmacodynamic measures. An attempt at linking ibuprofen pharmacokinetics with analgesic response found no significant difference in pain intensity between doses of 400mg, 600mg and 800mg despite roughly proportional increases in AUC. However, a new formulation of ibuprofen 400mg, which resulted in poor bioavailability to the extent that the AUC was roughly half that of the standard 400mg formulation, resulted in less than half the analgesic effect (Laska EM et al, 1986), suggesting a ceiling effect in the dose-response curve over 400mg.

As shown in Figures 1.3 and 1.4, systematic reviews of clinical studies on diclofenac and ibuprofen have similarly shown that dose-response seems to plateau within the usual dose range (McQuay HJ & Moore RA, 1998). Whilst number needed to treat for 50 percent pain relief does decrease with increasing diclofenac doses (25mg to 100mg) when corrected for placebo responders, the difference is not statistically significant and indeed negligible between 50mg and 100mg (McQuay HJ & Moore RA, 1998), suggesting a ceiling effect at around 50mg. Overall for post-operative pain, diclofenac 50mg seems to be as effective as intramuscular morphine 10mg, twice as effective as paracetamol 1g and tramadol 100mg, and eight-fold more effective than codeine 60mg.

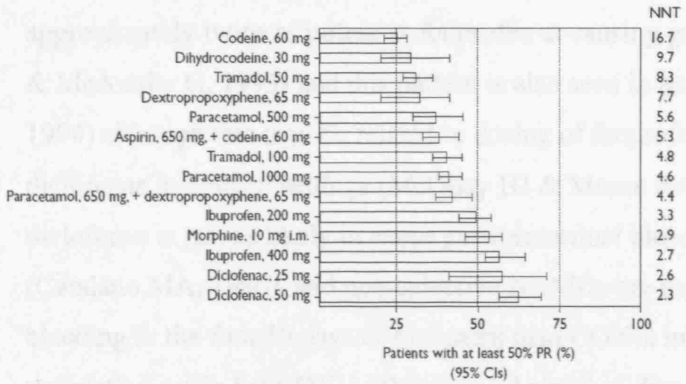


Figure 1.3.

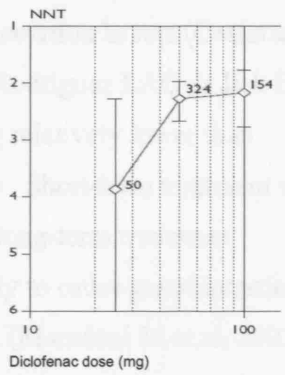


Figure 1.4.

Figure 1.3: Comparison of analgesics for peri-operative pain relief (PR) in adults.
Figure 1.4: Dose response for diclofenac using a fixed 19 percent placebo response rate. Numbers are patients given active treatments.
Figures reproduced from: McQuay HJ & Moore RA, 1998.

Using a combination of this clinical pharmacodynamic information, known pharmacokinetic properties, and the pharmacological mode of action of diclofenac, it is possible to propose a reasonable pharmacokinetic/pharmacodynamic relationship in the absence of a clear blood level/response correlation in the literature. The clinical studies used to derive the dose response relationship all used immediate release formulations of diclofenac (McQuay HJ & Moore RA, 1998). It has already been shown that absorption from such formulations can display large variability, which suggests that diclofenac efficacy is probably not related to attaining a maximal blood concentration, or if it is, that concentration is relatively low. Given that diclofenac's primary mode of analgesia is through inhibition of COX-2, and that diclofenac is a potent COX-2 inhibitor as shown by its time dependent rather than concentration dependent inhibition (Blobaum AL & Marnett LJ, 2007, Rowlinson SW et al, 2003), it would seem reasonable to assume that the pharmacodynamics of diclofenac are related to the AUC, which is a pharmacokinetic measure of drug exposure.

1.1.5.2 Adverse effects

Table 1.1 gives the incidence of adverse reactions seen with diclofenac. NSAID-induced serious upper gastrointestinal disease causes an estimated 12 000 emergency hospital admissions per year in the United Kingdom (Blower AL et al, 1997). Diclofenac is

approximately twice as potent as ibuprofen at causing gastric ulceration in rats (Cashman J & McAnulty G, 1995) and this pattern is also seen in humans (Rodriguez LAG & Jick H, 1994) although this may be related to dosing of ibuprofen being relatively lower than diclofenac in clinical settings (McQuay HJ & Moore RA, 1998). Short-term treatment with diclofenac is just as likely to cause gastrointestinal bleeding as long-term treatment (Catalano MA, 1986), and non-selective NSAIDs are more likely to cause gastrointestinal bleeding in the first 30 days of treatment than COX-2 inhibitors (Mamdani M et al, 2002), suggesting a role for COX-1 inhibition. Animal studies point to a possible explanation for the similar incidence of gastrointestinal bleeding in short-term and long-term treatment: COX-1 deficient mice have no gastrointestinal pathology (Morita I, 2002); a possible explanation being that prolonged inhibition of COX-1 could allow other pathways to compensate for the absence in 'housekeeper' prostaglandins produced by COX-1 in the gastrointestinal tract. As with most NSAIDs, it appears that gastrointestinal bleeding with diclofenac has some relation with dose, with 150mg per day or more posing the greatest risk (Lewis SC et al, 2002).

Allergic-type reactions may be more prevalent with NSAIDs than other drug classes; an analysis of adverse drug reaction reports found a relatively higher proportion of anaphylactic reactions with NSAIDs, in particular diclofenac, ibuprofen and naproxen, than with other drugs (van Puijenbroek et al, 2002). Hypersensitivity to NSAIDs usually manifests either in the airways or skin, but rarely both at the same time (de Weck AL at al, 2006). True anaphylactic shock occurring within a few minutes of drug exposure and mediated by immunoglobulin E (IgE) is relatively rare with NSAIDs, the more common hypersensitivity reactions occur between 30 minutes and a few hours post-dose. It is likely that basophil activation, either through NSAID direct action on the complement system or COX-mediated inhibition of prostaglandin E2 synthesis causing decreased inhibition of basophil mediator release, is the primary mechanism of these hypersensitivity reactions (Sanz ML et al, 2005). Prostaglandin E2 inhibits the production of sulphidoleukotrienes and other mediators of hypersensitivity from mast cells. The release of these sulphidoleukotrienes in response to NSAIDs may have a genetic component (de Weck AL at al, 2006). This genetic component is not a plausible explanation for most NSAID hypersensitivity reactions however. This is because a feature of these hypersensitivity

reactions is that they are often transient, and manifestation does not normally occur until adolescence or early adulthood (de Weck AL at al, 2006).

Table 1.1: Adverse drug reactions to diclofenac.

ADR Rates (*Diclofenac SPC*, 2005).		
Occasional (>1-10%)	Gastrointestinal	Epigastric pain, nausea, vomiting, diarrhoea, abdominal cramps, dyspepsia, flatulence, anorexia
	Central nervous system	Headache, dizziness, vertigo
	Skin	Rashes, skin eruptions
	Liver	Elevation of serum aminotransferase enzymes (ALT, AST)
Rare (>0.001%-1%)	Gastrointestinal	Gastro-intestinal bleeding (haematemesis, melaena, bloody diarrhoea), gastro-intestinal ulcers with or without bleeding or perforation
	Central nervous system	Drowsiness, tiredness
	Skin	Urticaria
	Liver	Liver function disorders including hepatitis (in isolated cases fulminant) with or without jaundice
	Kidney	Oedema
	Hypersensitivity	Hypersensitivity reactions (e.g. bronchospasm, anaphylactic/anaphylactoid systemic reactions including hypotension).
Isolated cases (<0.001%)	Gastrointestinal	Aphthous stomatitis, glossitis, oesophageal lesions, lower gut disorders (e.g. non-specific haemorrhagic colitis and exacerbations of ulcerative colitis or Crohn's proctocolitis, colonic damage and stricture formation), pancreatitis, constipation
	Central nervous system	Disturbances of sensation, paraesthesia, memory disturbance, disorientation, insomnia, irritability, convulsions, depression, anxiety, nightmares, tremor, psychotic reactions, aseptic meningitis
	Skin	Bullous eruptions, eczema, erythema multiforme, Stevens-Johnson syndrome, Lyell's syndrome, (acute toxic epidermolysis), erythroderma (exfoliative dermatitis), loss of hair, photosensitivity reactions, purpura including allergic purpura
	Blood	Thrombocytopenia, leucopoenia, agranulocytosis, haemolytic anaemia, aplastic anaemia.
	Hypersensitivity	Vasculitis, pneumonitis
	Cardiovascular system	Palpitations, chest pain, hypertension, congestive heart failure
	Special senses	Disturbances of vision (blurred vision, diplopia), impaired hearing, tinnitus, taste disturbances.

Percentage Incidence of Serious ADRs (Catalano MA, 1986)		
Peptic ulcer disease:	Short-term trials	0.16%
	Long-term trials	0.34%
Gastrointestinal bleeding:	Short-term trials	0.16%
	Long-term trials	0.17%
Hepatitis:	Short-term trials	None
	Long-term trials	0.26%
Thrombocytopaenia	Short-term trials	None
	Long-term trials	0.17%

Acute renal failure is a rare occurrence with NSAIDs, although it is potentially more likely in acute situations such as the peri-operative period, where precipitating factors such as

dehydration or concurrent nephrotoxic drug therapy may also be present. Transient, clinically unimportant reductions in glomerular filtration have been observed in patients with previously normal renal function given NSAIDs compared to placebo in the peri-operative period (Lee A et al, 2000). The primary mechanism of NSAID-induced renal dysfunction is probably COX-mediated decrease in blood flow to the juxtamedullary region through the inhibition of vasodilatory prostaglandins (Barkin RL & Buvanendran A, 2004). This distribution of blood flow is COX-1-mediated, and NSAID overdose can lead to renal papillary necrosis due to a mixture of decreased blood supply and direct cytotoxic effects of the drug, which tends to accumulate in the intrapapillary region (Whelton A, 2001). Despite this, clinical studies of COX-2 inhibitors have found similar adverse renal effects to non-selective NSAIDs (Brater DC et al, 2001). Both COX-1 and COX-2 are constitutively expressed in the kidney, their varying distribution suggesting differing function; COX-2 inhibition may be responsible for producing prostaglandins responsible for chloride resorption, subsequent activation of anti-diuretic hormone (ADH) leading to fluid retention (Whelton A, 2001).

The incidence of hepatotoxicity associated with diclofenac is approximately one to five cases per 100 000 patients, and acute hepatitis is the most common manifestation (Chitturi S & George J, 2002). Whilst serious hepatotoxicity is rare, approximately 15 percent of patients receiving diclofenac may get asymptomatic elevations in serum transaminases (Banks AT et al, 1995). Most cases of diclofenac-induced hepatotoxicity present one to six months after commencing treatment, with allergic-type fast-onset occurrence virtually unknown (Banks AT et al, 1995). The most likely cause of diclofenac-induced hepatotoxicity is the formation and subsequent accumulation of a toxic metabolite (Banks AT et al, 1995), possibly catalysed by a rare polymorphism in a metabolic enzyme. Whilst patients with diclofenac-induced hepatotoxicity were not found to have higher frequencies of non-wild-type CYP2C9 alleles (Aithal GP et al, 2000), a proposed mechanism has linked CYP2C9 with diclofenac hepatotoxicity in human liver microsomes (Yan Z et al, 2005). However, the major metabolites thought to be implicated are 1',4'- and 2,5-quinone imine derivatives of diclofenac (Tang W, 2003) and as the mechanism is yet to be elucidated, diclofenac induced hepatotoxicity remains idiosyncratic.

The final potential adverse effect of diclofenac is non-gastrointestinal bleeding. The *in vitro* ability of diclofenac to inhibit platelet aggregation is via COX-1 mediated thromboxane inhibition, and high-dose animal studies have shown marked decreases in thrombus formation (Todd PA & Sorkin EM, 1988). This has led many clinicians, and especially surgeons, to believe that NSAIDs pose a risk of bleeding complications. Fairly recent human studies have found that ibuprofen inhibits platelet function, as measured by the 'platelet-function analyzer-100', forty minutes after the final dose of a week-long course in seven out of 11 patients (Goldenberg NA et al, 2005), and that diclofenac 150mg per day decreases thromboxane biosynthesis in healthy volunteers (Tegeder I et al, 1998). The crucial element missing from these studies is a direct measure of bleeding time. Systematic review of studies on NSAIDs, which investigated blood loss in tonsillectomy, a high bleeding risk procedure, found NSAIDs did not increase bleeding complications (Cardwell M et al, 2005). This review included studies on ketorolac, which preferentially inhibits COX-1 over COX-2 (Cryer B & Feldman M, 1998). In addition, patients suffered less nausea and vomiting than in opiate comparator groups. It has therefore become clear that at standard therapeutic doses, diclofenac is unlikely to inhibit platelet function to the extent of causing clinically significant bleeding.

1.2 Paediatric Clinical Pharmacology

The majority of clinical studies referred to in the first part of this chapter have been conducted in adults. Indeed, it is routine for the exclusion criteria of clinical drug studies to stipulate that participants must be over a certain age, usually 16 or 18 years old. Apart from complications such as the requirement for parental consent, a major reason that children are excluded from clinical trials is that, from a drug-handling perspective, they form a heterogeneous group, which is usually subdivided into (*ICH Guideline*, 2000):

- Premature/preterm neonate
- Neonate (birth to 1 month)
- Infant (1 month to 2 years)
- Child (2 to 12 years)
- Adolescent (12 to 18 years)

1.2.1 Drug Handling in Children

At birth, gastric pH is approximately neutral but falls to 1.5 to 3 within the first few hours of life (Morselli PL et al, 1980). Immaturity of gastric acid secretory mechanisms means that the stomach pH rises to 4 or above, gradually returning to adult values by two years of age (Morselli PL et al, 1980, Kearns GL et al, 2003, Sreedharan R & Mehta DI, 2004). It has been proposed that the decreased absorption of acidic drugs such as rifampicin, phenytoin and nalidixic acid in neonates and infants could be due to increased gastric pH (Morselli PL et al, 1980).

The large surface area of the small intestine relative to the stomach means this is where most drug absorption takes place (Washington N et al, 2002). Gastrointestinal surface area is adult-equivalent by 20 weeks gestation (Kearns GL et al, 2003) and although in the neonate relative immaturity of the gastrointestinal mucosa has been hypothesised to cause increased drug absorption (Kearns GL, 2000), membrane transport by passive diffusion and active transport reach adult levels by four to six months (Kearns GL et al, 2003, de Zwart LL et al, 2004).

Gastrointestinal motility can affect the rate, and occasionally extent, of drug absorption. A high gastric emptying rate is seen in children under six months old (Morselli PL et al, 1980), although this then slows relative to adult values in infants and children (de Zwart LL et al, 2004). The number of high amplitude propagated contractions of the colon, and therefore bowel movements, decreases between the ages of one and 17 years (Di Lorenzo C et al, 1995). Decreased lower gastrointestinal transit time could potentially decrease the extent of drug absorption in infants and children (Gilman JT, 1990, Kearns GL, 2000), although slower gastric emptying could balance this. Milk feeds can conjugate drugs and hence decrease the extent of drug absorption (Gilman JT, 1990) and it has been proposed that as neonates and infants have an almost continuous presence of milk in the stomach, the absorption of lipophilic and protein-bound drugs is potentially decreased (de Zwart LL et al, 2004). Other factors which may affect paediatric oral drug absorption include: changes in gut flora (Linday L et al, 1987); immaturity of conjugation and transport of bile salts decreasing lipophilic drug absorption (Kearns GL et al, 2003); and febrile illness and other disease states such as gastroenteritis causing erratic drug absorption (Morselli PL et al,

1980, de Zwart LL et al, 2004). In general, the overall rate of oral drug absorption is potentially decreased in infants and children, with the extent remaining unchanged for all but fat-soluble compounds (de Zwart LL et al, 2004).

Rectal drug absorption is efficient in infants and children (Morselli PL et al, 1980) and drugs such as diazepam and paracetamol exhibit higher bioavailability in neonates and young infants (Kearns GL, 2000). This is probably due to immaturity of hepatic metabolism as opposed to enhanced permeability of lower gastrointestinal mucosa (Kearns GL et al, 2003). Low skeletal muscle mass, variable blood flow to the muscles and reduced muscle contractility make drug absorption erratic and unpredictable (Morselli PL et al, 1980, Kearns GL et al, 2003) and the pain involved in administering intra-muscular injections also makes this route unsuitable for younger patients.

Physiological changes during development, especially in the first year of life, mean that the distribution of drugs may not approximate adult values. Total body water is high at birth and gradually decreases to adult equivalent levels by 12 years (Friis-Hansen B, 1971). Body fat increases from 15 to 30 percent of body weight during the first year of life. In girls, body fat tends to remain at this level but boys tend to lose body fat during puberty (Friis-Hansen B, 1971). Furthermore, the adipose tissue of neonates contains a higher proportion of water than that of adults (Kearns GL, 2000). The higher total body water in infants and children may increase the V_D of drugs distributed in body water (de Zwart LL et al, 2004) with consequent decreased plasma concentrations than would be expected when dosed by body weight (Kearns GL et al, 2003).

Drugs that bind to plasma proteins tend to have a low V_D. Paediatric patients exhibit lower protein binding than adults (Grandison MK & Boudinot FD, 2000) but this may only be true for neonates and young infants. Total circulating plasma protein concentrations are decreased in neonates and young infants (Kearns GL et al, 2003) reaching adult equivalent levels by one year, leading to an elevation in the free fraction of highly-bound drugs in neonates and young infants (de Zwart LL et al, 2004). However, an increase in free fraction is rarely significant; whilst a higher proportion of drug in circulation may be unbound, the concentration of unbound drug is unlikely to change unless elimination is

saturated (Benet LZ & Hoener BA, 2002). It is for this reason that measurement of free phenytoin concentration should be routine in clinical practice, as decreased total phenytoin in patients with low albumin levels does not necessarily mean free-phenytoin concentrations are low (Nation RL et al, 1990).

Several organs express metabolising enzymes with the liver being the major site of drug metabolism. Neonates and infants have a larger liver volume in relation to body weight compared with adults (Johnson TN et al, 2005, Morselli PL et al, 1980), and although liver volume could be a determinant of metabolising capacity, the mechanism of metabolism is also an important factor in age-related changes in metabolic activity (Murry DJ et al, 1995). Despite its relative size, the neonatal and infant liver contains 20 percent fewer hepatocytes than the adult liver, and the hepatocytes are approximately half the size. Hepatocellular maturation appears to be complete by early adolescence (Alcorn J & McNamara PJ, 2002). The adult liver acinus can be divided into three zones, the functionality of which probably develops as an adaptive response to the varying blood composition as it passes from the periportal to pericentral acinus. Foetal liver acinus function is not as well defined, the functional zones developing with age (Alcorn J & McNamara PJ, 2002). The importance of this difference in the neonate, infant and child's liver in terms of drug metabolism is yet to be fully understood.

The development of phase I enzymes, such as cytochrome P450 (CYP), has been extensively studied. The total CYP enzyme content of foetal liver is approximately 30 percent that of the adult value, and reaches 50 percent by nine months (Treluyer JM et al, 1996). It is thought that adult-equivalent levels are achieved by ten years of age (Hines RN & McCarver DG, 2002). The expression of individual CYP subtypes can vary during development and this is further confounded by inter-individual variability that can occur. A summary of the developmental changes in phase I enzymes is given in table 1.2. Phase II metabolism has been less well studied in children although it is plays a major role in the metabolic pathway of many drugs. Glutathione-S-transferases (GST) conjugate glutathione to a wide range of electrophilic or reactive lipophilic agents and alkylating agents (Alcorn J & McNamara PJ, 2002). Examples of the developmental changes in GST include GSTP1 which is highly expressed in foetal hepatic samples but undetectable in adults, GSTA1 and

GSTA2 which increase to adult levels during the infant period and GSTM which is expressed at adult levels at birth (McCarver DG & Hines RN, 2002). N-Acetyltransferase (NAT), the enzyme catalysing acetylation, reaches adult levels after one year of age (Alcorn J & McNamara PJ, 2002) and epoxide hydrolase (EPHX), the enzyme catalysing trans-dihydrol derivative production, appears to have similar activity in neonates and adults (McCarver DG & Hines RN, 2002).

Table 1.2: Ontogeny of major hepatic drug metabolising enzymes.

Enzyme	Neonate	Infant	Child	Adult	Comments
CYP1A1	+	-	-	-	Not expressed in adult liver.
CYP1A2	-	+	+	+	10% at 4weeks, 50% at 1year, approaches adult value after 1 year.
CYP1B1	+	+ (EH)	+ (EH)	+ (EH)	Mainly expressed extrahepatically, not expressed in adult liver.
CYP2A6	?	+	+	+	Not expressed in foetal liver, adult levels by 1 year.
CYP2B6/7	?	+	?	+	10% of adult levels by 1 year but shows high interindividual variability.
CYP2C (total)	+	+	+	+	30% adult levels at 1week to 1 year, adult levels reached at some point after 1 year.
CYP2C9	+	+	+	+	Adult levels by 5 months.
CYP2C19	+	+	+	+	Highly variable between 5months and 10 years, adult value by 10 years.
CYP2D6	+	+	+	+	Adult levels by 3 to 5 years.
CYP2E1	+	+	+	+	Adult levels by 1 year.
CYP3A4	+	+	+	+	30% of adult levels at 1 month, may only reach adult levels in adolescents.
CYP3A5	+	+	+	+	Highly variable, independent of age.
CYP3A7	+	+	-	-	High neonatal activity, not expressed in adults - CYP3A4 takes over as major CYP3A.
FMO1	+	-	-	-	Rapid decline after birth, no activity in adults.
FMO3	-	+	+	+	Expression starts during 1st year, adult levels by 11 to 18 years.
ADH1	+	-	-	-	Not expressed in adult liver.
ADH2/3	+	+	+	+	Adult levels by 1 to 2 years.

+ activity or protein detected; - no detectable activity or protein; ? not determined/conflicting data; CYP cytochrome P450; FMO flavin-containing monooxygenases; ADH alcohol dehydrogenase; EH extrahepatic.

Data obtained from:
Alcorn J & McNamara PJ. 2002, de Zwart LL et al, 2004, Hines RN & McCarver DG, 2002, Koukouritaki SB et al, 2004, Treluyer JM et al, 1996

Several uridine 5'-diphosphate glucuronosyltransferases (UGTs) have been characterised although little is known about developmental aspects of their expression (De Wildt RM et al, 1999). However, some drugs provide an insight into the development of glucuronidation. Grey baby syndrome in neonates, caused by high serum and tissue levels of chloramphenicol, is thought to be due to an inability to conjugate the drug (McCarver DG & Hines RN, 2002). Ibuprofen (24-fold), amitriptyline (16-fold) and buprenorphine (12-fold) are examples of drugs found to have a lower degree of glucuronidation in paediatric liver samples compared with adults (Strassburg CP et al, 2002). Sulfotransferases (SULT) are generally well expressed in the neonate as sulfation may be important for detoxification in the foetus (Alcorn J & McNamara PJ, 2002). However, SULT1C1 has been found to be expressed in much higher concentrations in adult tissues than foetal samples, suggesting there may be developmental aspects to some sulfation enzymes (McCarver DG & Hines RN, 2002).

Drug metabolism also takes place in other tissue such as the gastrointestinal tract and the lung, although the ontogeny of this metabolism has not been extensively studied. Similar to its hepatic expression, significantly lower CYP3A4 expression has been seen in neonatal duodenal tissue compared with children over five years (Johnson TN et al, 2001). Aryl hydrocarbon hydroxylase activity was found to be lower in small intestine samples of eight month old children compared with 18 year olds although no differences in EPH or GST were seen (Stahlberg MR et al, 1988). This highlights the point that there may well be developmental differences in tissues other than the liver, which may affect metabolism in children.

Most water-soluble drugs and their metabolites are eliminated by the kidneys. The major mechanism of drug clearance is glomerular filtration although some undergo active tubular secretion. Nephrogenesis is complete by 36 weeks gestation, although glomerular filtration rate (GFR) is only five percent of the adult value (Alcorn J & McNamara PJ, 2002). Although renal tubular development continues throughout childhood, renal blood flow and GFR are generally thought to reach adult-equivalent levels by one year (Kearns GL et al, 2003). However, the clearance of *p*-aminohippurate (PAH) does not reach adult-equivalent values until two years of age and this may be an underestimation, as PAH is not exclusively

cleared by the kidneys in neonates and infants as it is in adults (Alcorn J & McNamara PJ, 2002). Whilst renal tubular maturation is complete by one year, little is known about the ontogeny of drug carrier systems in the tubular epithelium. Furthermore, infant urinary pH values are generally elevated which may influence the reabsorption of weak acids and bases (Alcorn J & McNamara PJ, 2002), so the renal drug clearance may well continue to develop after one year. Clearance of high extraction ratio drugs dependent on hepatic blood flow should not be altered in paediatric patients. Any developmental changes in hepatic blood flow are thought to be insignificant for drug clearance in relation to immaturity of metabolic enzyme expression (Alcorn J & McNamara PJ, 2002).

Very few studies have investigated the ontogeny of drug-receptor interactions and pharmacodynamics in children. However, there is evidence that age-related differences occur in drug-receptor interactions of warfarin and cyclosporin, and differences in clinical response to the same plasma level of midazolam in different age groups occur (Kearns GL et al, 2003).

1.2.2 Paediatric Dosing Strategies

The simplest way of determining dosing for a paediatric patient is by age. As seen earlier in this chapter, maturation of processes involved in drug handling are linked with age, and for wide therapeutic-index over-the-counter medicines, age is often the most appropriate measure. For most prescribed medicines, whose therapeutic index may be narrower or where therapeutic effect needs to be optimised, doses are usually scaled by body weight (*BNFC*, 2006). It is well recognised that dosing by body weight leads to potential under-dosing in infants and young children, as drug clearance often exceeds body-weight normalised predictions (Holford NHG, 1996)

Galileo was possibly the first scientist to recognise that metabolic processes were more likely to correlate with body surface area rather than volume (McMahon T, 1973). Heat production rate divided by body surface area measurement was found to be constant in various sized dogs as early as 1883 (Kleiber M, 1947). Compared with body weight, body surface area was thus thought to provide a more accurate measure of drug clearance, and

therefore dosage in paediatric patients (Cawford JD et al, 1950), a belief that currently persists in clinical practice (*BNFC*, 2006, Lack JA & Stuart-Taylor ME, 1997).

Several studies in the 1930s looked at the relationship between basal metabolic rate, usually measured by heat generation, and body weight of various sized animals. The surprising finding was that across a number of mammal species, a plot of Log basal metabolic rate versus Log weight yielded a straight line with a slope of ¾. Body surface area can be approximated using weight$^{2/3}$ ($w^{2/3}$), whereas these studies suggested the relationship to be $w^{3/4}$ (Kleiber M, 1947). This allometric $w^{3/4}$ relationship has been found to scale metabolic processes from the subcellular level to the ecosystem, providing an as-yet unexplained unifying scale of metabolic processes with size (West GB & Brown JH, 2005). If basal metabolic rate is directly proportional to body size, the power should be a multiple of 1/3, as size is measured in three dimensions. The 'fourth dimension' in scaling of metabolic rate versus weight has been explained with theories such as: elastic criteria relating muscle size (which is proportional to $w^{3/8}$) and muscle diameter (d^2) such that maximal power output $\propto (w^{3/8})^2 = w^{3/4}$ (McMahon T, 1973); the fractal nature of biological structures causing the appearance of a fourth dimension (West GB et al, 1997, West GB et al, 1999); optimality of transport systems in removing toxins (Banavar JR et al, 1999, Dreyer O & Puzio R, 2001); and manipulation of Poiselle's law of fluid transport through a cylinder (Rau ARP, 2002). It could also be argued that field metabolic rate would be a more appropriate measure than basal metabolic rate when trying to correlate dosing in hospitalised or sick individuals. In mammals basal metabolic rate actually scales closer to $w^{0.734}$ but this was rounded to 0.75. In fact, the field metabolic rate (measured under active rather than resting conditions) in mammals is probably closer to 0.75 than basal metabolic rate (Nagy K, 2005).

Despite the difficulty in explaining the ¾ power laws over the previous 75 years, the basic observation remains. Probably the main reason that human narrow therapeutic-index drug dosing has adhered to the body surface area method, is that a nine-fold difference in weight is required to see a significant difference in predicted basal metabolic rate from surface area ($w^{2/3}$) and $w^{3/4}$ (Kleiber M, 1947). It has recently been suggested that allometric scaling of dosing for paediatric patients over eight years old should be investigated (Kearns GL, 2003)

and a growing number of pharmacokineticists are advocating the use of allometric $w^{3/4}$ scaling for modelling clearance in human studies in order to delineate body size from other maturational processes (Holford NHG, 1996, Anderson BJ et al, 2006, Meibohm B et al, 2005). This makes two major assumptions: that drug clearance is proportional to basal metabolic rate, and that basal metabolic rate scales to $w^{3/4}$ within a species.

Whilst it may be reasonable to assume that removal of toxins (drug clearance) is linked with metabolic rate, it is less clear whether basal metabolic rate scales to $w^{3/4}$ within a species, as nine-fold weight ranges are required to see significant differences with other potential exponents such as $w^{2/3}$ (Kleiber M, 1947). Whilst it could be argued that the $w^{3/4}$ relationship has been found at the subcellular level, and therefore points to a unifying theory in the organisation of biological structures (West GB & Brown JH. 2005), it should also be noted that no juvenile organism appears to have been included in the original observations. Basal metabolic rate does increase during development, but whether it scales to $w^{3/4}$ is not possible to ascertain from published studies as they tend to look for more complex relationships and use age and sex adjusted measures of body size (Cole TJ & Henry CJK, 2005). Certainly scaling of clearance to $w^{3/4}$ fails to predict drug clearance in neonates (Allegaert K et al, 2005), the remaining variability thought to be due to maturational processes (Anderson BJ et al, 2006, Meibohm B et al, 2005). It must therefore be noted that using $w^{3/4}$ in paediatric dosing schemes is a controversial issue, and is yet to attain widespread acceptance. However, a growing number of biological observations do support the $w^{3/4}$ theory. For example, infants appear to have higher liver volumes compared with their size, and a relationship shown in equation 1.1 has been derived from a large pool of anatomical data from birth to age 18 years (Johnson TN et al, 2005):

$$LV = 0.722 \times BSA^{1.176} \qquad \text{Equation 1.1}$$

Where:

LV = Liver volume.
BSA = Body surface area.

As BSA scales approximately with $w^{2/3}$ it can be seen that liver volume scales with $w^{0.78}$ (= $(w^{0.67})^{1.176}$), providing further support to the theory that some underlying biological factor makes metabolic processed scale with approximately $w^{0.75}$.

1.2.3 Licensing of Paediatric Medicines

The Medicines Act of 1968 was introduced following the thalidomide disaster; thalidomide was used by pregnant women for morning sickness and caused a high proportion of limb defects (phocomelia) in their babies. Subsequently all medicines were required to undergo formalised testing overseen by the Medicines and Healthcare products Regulatory Agency (MHRA), formally known as the Medicines Control Agency (Choonara I & Dunne J, 1998). The MHRA then issues a product license to the pharmaceutical company developing a medicine, the process of obtaining which is shown in figure 1.5.

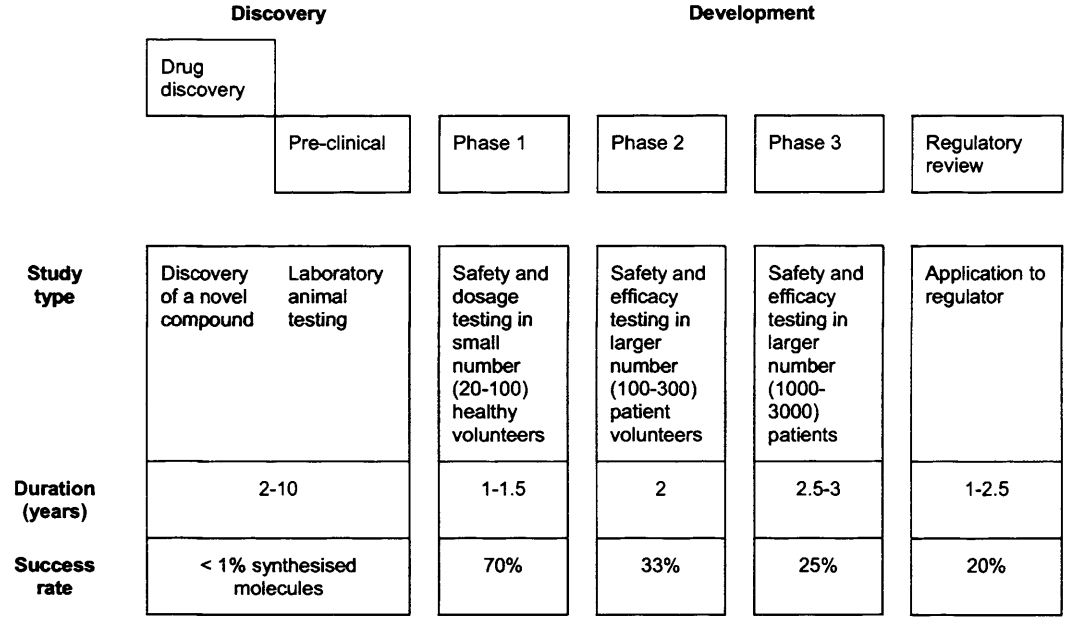


Figure 1.5: Process of obtaining a medicinal product license.
Figure reproduced from: *ABPI*, 2000.

Whilst the licensing process provides assessment of efficacy, safety and quality of a medicine for a specific indication, there is no stipulation that doctors must prescribe according to the product license ('off-label') or even that they cannot prescribe a medicine without a product license. Factors such as a small market potential, the need for obtaining parental consent for undertaking clinical trials and perceived difficulties in carrying them out, and the complexity and expense of developing paediatric formulations, mean that many medicines used for children are not licensed (Turner S et al, 1998). The majority of such

drugs are 'off-label' in that they are licensed but efficacy, safety and dosing for children have not been assessed formally by the regulatory authorities. Furthermore, with 'off-label' medicines, formulations usually have to be altered (tablets crushed, capsules opened) or extemporaneous formulations produced, rendering the use in children as unlicensed. The issue of unlicensed medicine use in children is prevalent throughout the world (Conroy S et al, 2000, t'Jong WT et al, 2000).

There is a clear need to undertake clinical trials of previously untested medicines in children. The example of paediatric acute lymphoblastic leukaemia (ALL) highlights how clinical trials on a condition treated largely with medicines can improve survival. Co-ordinated multi-centre trials led to an improvement in five-year survival from 37 to 70 percent of children with ALL from 1971 to 1985 in the UK, a trend that was also seen in the USA (Caldwell PHY et al, 2004). The use of unlicensed medicines risks therapeutic failure due to poor bioavailability (Notterman DA et al, 1986), there is growing evidence that unlicensed medicines may cause more adverse drug reactions (Choonara I & Conroy S 2002, Turner S et al, 1999), and the propensity for medication errors due to manipulation of high-strength adult formulations is also increased (Koren G et al, 1986). The 1997 Food and Drug Administration Modernisation Act, giving financial incentives for paediatric medication research in the form of extended patents, has led to an increase in the number of safety, efficacy and pharmacokinetic studies in children undertaken in the USA. New, unpredicted, paediatric dosing and safety information was found for 36 percent of drugs studied between 1998 and 2001 (Roberts R et al, 2003). Similar legislation has now been implemented across Europe, a particular feature of which allows for the licensing of old, off-patent medicines by niche-market drug companies who can develop and test a paediatric formulation (Stephenson T, 2006).

One of the most compelling arguments for the licensing of medicines specifically for children is that it will lead to the development of a paediatric formulation. Using unlicensed extemporaneous formulations can cause therapeutic failure (Notterman DA et al, 1986). Giving tablets to children is problematic as many are unable to swallow them whole even with specific training (Czyzewski DI et al, 2000). Despite this, children often have to be given tablets or capsules due to a lack of appropriate liquid formulations (Schrim

E et al, 2003). Splitting tablets can cause dose inaccuracies (Teng J et al, 2002), and crushing tablets and mixing with food can impair drug release and absorption (Breitkreutz J et al, 1999). Until recently the need for paediatric formulations with tested bioavailability was largely ignored by authors and journal editors involved in paediatric clinical trials, with most providing no or inadequate information on how the drug was administered (Standing JF et al, 2005).

1.3 Diclofenac for Acute Pain in Children

The belief that neonates and infants do not suffer pain due to neurological under development resulted in avoidance of the use of potent analgesic agents (Beyer JE & DeGood DE, 1983, Mather L & Mackie J, 1983). The biology of pain does show developmental differences between neonates and adults in both peripheral and central inflammatory response (Fitzgerald M, 1995) but paediatric anaesthetists have long recognised that neonates do suffer from painful stimuli (Purcell-Jones G, 1988). As preverbal neonates and infants can show physiological signs of pain, and by three years of age children are able to indicate the presence and severity of pain, it is recommended that pain is anticipated, prevented or controlled in all age groups (Morton NS, 1999). Modern paediatric analgesic techniques often take a multimodal approach employing local anaesthetics, opiates and NSAIDs such as diclofenac (Cunliffe M & Roberts SA, 2004, Howard RF, 2003, Lloyd-Thomas AR, 1999). NSAIDs are particularly useful for day-case surgery where their potency can minimise the use of sedating opiates and allow prompt discharge (Morton NS, 1999).

Diclofenac was found to be the most commonly used unlicensed medicine on the paediatric surgical ward (Turner S et al, 1998, Conroy S & Peden V, 2001). This constituted mainly of 'off-label' use as the suppositories are licensed for juvenile arthritis, but where a proportion of a soluble tablet was given, the use would be unlicensed. In 2006 diclofenac suppositories became licensed for acute pain from age six years and over; the reason for the age not being down to one year or less, reflecting clinical practice, is probably because there is little pharmacokinetic data to support dosing in this age-group.

Developmental differences mentioned earlier in this chapter mean that diclofenac pharmacokinetics may differ between adults and paediatric patients. Absorption rate could be altered in neonates and infants due to their higher gastric pH (Kearns GL et al, 2003, Morselli PL et al, 1980) meaning diclofenac may dissolve into solution higher up in the gastrointestinal tract. As diclofenac is highly plasma-protein bound (Davies NM & Anderson KE, 1997), the extent of absorption could theoretically decrease when given with milk feeds, as with phenytoin (*BNFC*, 2006), but no confirmatory or contrary information could be found in the literature on diclofenac pharmacokinetics.

Diclofenac's plasma protein binding mainly to albumin has been reported to be greater than 99.5 percent (Davies NM & Anderson KE, 1997). Whilst lower concentrations of plasma proteins seen in neonates and infants (de Zwart LL et al, 2004) may to affect the protein bound fraction, there will be no differences in free diclofenac concentration (Benet LZ & Hoener BA, 2002). Higher proportions of body water in infants compared with adults (Kearns GL et al, 2003) may cause diclofenac to have a higher apparent V_D by weight in this group of patients.

Only a single study has investigated the maturation of CYP2C9, and it found adult equivalent protein levels by five months of age although diclofenac 4'-hydroxylase activity may not be adult-equivalent by this age (Koukouritaki SB et al, 2004). This means that theoretically infants, children and even adolescents could be less able to metabolise diclofenac to 4'-hydroxydiclofenac, but the impact of this on overall diclofenac clearance is uncertain as other metabolic pathways are also involved (Tang W, 2003.). Diclofenac phase II metabolism is mainly through glucuronidation (Todd PA & Sorkin EM, 1988), which may be impaired in neonates and infants (de Zwart LL et al, 2004). If phase II metabolism is altered in children, it would seem most likely that diclofenac is inefficiently glucuronidated, although sulphation may compensate as is the case with paracetamol. As weak acids, elevated urinary pH will mean diclofenac and its hydroxy metabolites are less likely to undergo tubular reabsorption in neonates and infants and so may be excreted more efficiently. However, as renal drug excretion may not be fully mature in neonates and infants, and as diclofenac's pharmacological action may decrease renal blood flow via prostaglandin inhibition, it seems most likely that diclofenac elimination will be decreased.

Biliary excretion accounts for a relatively small proportion of diclofenac clearance (Davies NM & Anderson KE, 1997), so the overall effect of decreased biliary function in neonates and infants may not significantly affect diclofenac elimination by this route.

A single published study including the pharmacokinetics of 25mg enteric-coated tablet of diclofenac in seven children, mean weight 16kg, with juvenile arthritis (Haapasaari J et al, 1983) showed a similar AUC range to that reported in adults given 50mg enteric-coated tablets (Davies NM & Anderson KE, 1997), and maximum concentrations were highly variable. This suggests a reasonable dose to approximate to 50mg in adults is 1.5mg/kg, although the small sample size of seven patients means this is not necessarily reflective of the paediatric population with juvenile arthritis. The remaining studies have focussed on peri-operative diclofenac use. A study of 20 children given 2mg/kg of diclofenac rectally (Murphy DB et al, 2000) found relatively high AUC, being similar to 100mg suppository in adults (Davies NM & Anderson KE, 1997), and high maximum plasma concentrations possibly indicating relatively efficient rectal absorption in children. Intravenous doses of 0.5mg/kg of diclofenac were given to 10 children aged four to six with non-compartmental pharmacokinetic analysis showing a higher V_D and clearance than in adults when scaled by body weight (Korpela R & Olkkola KT, 1990). Higher V_D in children could be caused by them having proportionally more body water than adults, or it could also be due to V_D being calculated from weight scaled clearance (V_D = Ke/CL), which as shown earlier usually scales to a power function on weight which is less than one, meaning that linear weight scaled estimates overestimate clearance. An ambitious randomised trial comparing 1-2mg/kg of diclofenac in 11 children with paracetamol in 10 children attempted to find a concentration-effect relationship with diclofenac enteric-coated tablets and poker-chip pain scores (Romsing J et al, 2001). Whilst no significant correlation was seen with plasma diclofenac concentrations and pain scores, mean pain scores did decrease over the five hours post-dose, with the most consistent reduction at three and a half and four hours. The V_D and clearance of diclofenac were higher than typical adult values (Davies NM & Anderson KE, 1997) when scaled to body weight and AUC was similar to that seen with 50mg enteric-coated tablets in adults. The final study detailing diclofenac pharmacokinetics in children pooled data on enteric-coated tablets from a previous study (Romsing J et al, 2001) with 26 children given 1-2mg kg using an extemporaneous

suppository formulation (van der Marel CD et al, 2004). A pooled population pharmacokinetic approach was taken to determine the pharmacokinetics of diclofenac, 4'-hydroxydiclofenac, and 5-hydroxydiclofenac. Clearance of diclofenac normalised to an allometric $w^{3/4}$ model was the same throughout the different age groups but clearance scaled by weight alone was predicted to be 30 percent higher in children aged one to three compared with 12 to 16 year olds.

Descriptive pharmacokinetic data on diclofenac in children shows that weight scaled clearance and V_D tend to be higher, especially in younger age groups, with AUC achieving similar values to 50mg in adults at approximately 1.5mg/kg. Making dose predictions from the available data is problematic as four different formulations were used by three different routes in only 74 children aged two to 16 years. Furthermore, little information is provided in one of the suppository studies only published in abstract form (Murphy DB et al, 2000) and the juvenile arthritis study (Haapasaari J et al, 1983) in which the enteric-coated tablet pharmacokinetics only form a small section. Compared with weight-normalised clearance values in the published data suggesting that younger children may require a higher dose by body weight, it is difficult to see how the current recommended dose of 1-2mg/kg/day in divided doses (*BNFC*, 2006, *Diclofenac SPC*, 2005) was arrived at. In published clinical studies, there is a five-fold difference in the dose of diclofenac being used for acute pain: 0.5mg/kg (Tay CLM & Tan S, 2002), 1mg/kg (Mendham JE & Mather SJ, 1996), 2mg/kg (Nishina K et al, 2000), 2.5mg/kg (McGowan PR et al, 1998) highlighting the confusion on the optimum dose to use in clinical practice.

Despite the lack of published pharmacokinetic information to support current dose guidelines, diclofenac has been found to be effective in acute peri-operative pain. It decreases pain scores, opioid requirements and the need for supplementary analgesia such as paracetamol (Romsing J & Walther-Larsen S, 1997). The majority of studies on diclofenac for acute pain in children are from the peri-operative period, where it is most commonly used (Turner S et al, 1998), and focus on the efficacy of diclofenac (Romsing J & Walther-Larsen S, 1997). It is well recognised that randomised controlled trials designed to assess efficacy tend to be poor at reporting adverse reactions (Ernst E & Pittler MH, 2001). There are very few published trials specifically looking at the safety of diclofenac

for acute pain in children. A recent Cochrane systematic review included studies on diclofenac used for children in the peri-operative period following tonsillectomy and found no increase in bleeding compared with placebo (Cardwell M et al, 2005). A bronchoprovocation test in 72 asthmatic children found no decrease in forced expiratory volume following diclofenac administration (Short JA et al, 2000), but the bronchoprovocation method used was not ideal (Debley JS et al, 2005, Stevenson DD et al, 2006) and as only 72 children were recruited, the incidence of diclofenac-induced bronchospasm in asthmatic children could still be as high as one in 24 (maximum incidence=n/3=72/3≈24/1) (Hanley JA & Lippman-Hand A, 1983). In addition to the sparse literature on acute use of diclofenac and adverse reactions in children, the fact that it is largely used off-label means there is no formal post-market surveillance in this group and spontaneous reporting such as the MHRA Yellow Card Scheme may be less likely to receive reports (Conroy S et al, 2000)

There is also currently no licensed paediatric oral formulation of diclofenac (*BNFC*, 2006). The adult strength (50mg) soluble tablets can be used, as dose uniformity when they are dissolved in a fixed volume of water is likely to be good given the high solubility of diclofenac at neutral pH (*Codex*, 1993). However, such extemporaneous manipulations may cause dosing errors, especially if parents are asked to administer the dose at home (Koren G et al, 1986), and furthermore diclofenac tastes very bitter (personal experience) which may affect compliance. The enteric-coated tablets (25mg or 50mg) are less suitable for acute pain given the high variability in absorption lag (Chan KKH et al, 1990, Idkaidek NM et al, 1998, Lotsch J et al, 2000, Willis JV et al, 1979); many children are unable to swallow solid dose forms (Czyzewski DI et al, 2000), and suppositories are generally not well accepted by patients (Vyvyan HAL & Hanafiah Z, 1995).

1.4 Conclusions from Literature Review

Diclofenac is clearly an effective analgesic for acute pain in both adults and children; despite being unlicensed for acute pain in children, diclofenac is commonly used. There are several theoretical reasons to suggest that drug handling in paediatric patients may differ from adults, but the significance of these differences may be small compared with the large magnitude changes in body size that occur during development. Adverse drug

reactions and optimum dosing of many medicines for children have been sparsely studied, underlining the clear need to assess both safety and dosing of diclofenac for acute pain. The safety of diclofenac in children may not have been formally assessed for licensing purposes, or studied in multiple high-quality published trials, but it is widely used in children. This suggests that any safety problems such as large increases in known adverse reactions, or child-specific new adverse reactions such as grey baby syndrome with chloramphenicol, would be likely to have already come to light during clinical practice. Whilst systematic investigation of diclofenac safety in children remains important in order to characterise and quantify its adverse reactions in children to inform clinical practice, the development of paediatric dosing guidelines of a suitable oral formulation are most urgently required.

Rosemont Pharmaceuticals have developed a new oral liquid formulation of diclofenac sodium 50mg/5mL. It is a suspension with low pH meaning diclofenac is largely in solid form, and flavouring agents also contribute to masking the bitter taste. A bioequivalence study in healthy adult volunteers (unpublished) has shown the suspension to be bioequivalent to diclofenac soluble tablets. Whilst linked pharmacokinetic/pharmacodynamic studies of diclofenac have so far failed to link blood concentrations with effect, clinical studies have shown that 50mg provides the best balance between toxicity and efficacy in adults.

Only a single study *in vitro* has investigated the expression of CYP2C9 during development and no studies have sought to investigate CYP2C9 maturation through the use of probe drugs. Undertaking a pharmacokinetic study on diclofenac in children presents an ideal opportunity to determine whether CYP2C9 expression is adult-equivalent by one year of age (Koukouritaki SB et al, 2004). A potential confounding factor in such a study could be CYP2C9 genotype, and so it is important that this is accounted for when investigating age and diclofenac:4'-hydroxydiclofenac ratio. There are conflicting reports on the influence of CYP2C9 genotype and diclofenac clearance to 4'-hydroxydiclofenac *in vivo*, and none of the published studies have looked at polymorphisms beyond *2 and *3.

1.5 Proposed Research

1.5.1 Aims

1. To investigate the pharmacokinetics of diclofenac suspension in children and produce dosing guidelines for its use in acute pain.
2. To investigate the frequency and types of adverse drug reactions of diclofenac when used for acute pain in children, and compare with adult data.
3. To investigate the ontogeny of CYP2C9 expression accounting for CYP2C9 genotype.

1.5.2 Objectives

1. Conduct a clinical pharmacokinetic study on diclofenac suspension for acute pain in children including measures of 4'-hydroxydiclofenac and CYP2C9 genotype.
2. Conduct a prospective observational clinical study to ascertain the type and incidence of common adverse drug reactions to diclofenac when used for acute pain in children.
3. Conduct an epidemiological-type study to ascertain the type and incidence of less frequent adverse drug reactions to diclofenac when used for acute pain in children.

1.5.3 Overview and Rationale of Methods

1.5.3.1 Determining the Developmental Pharmacokinetics of Diclofenac Suspension

Rosemont Pharmaceuticals have already conducted a bioequivalence study (unpublished) in which 30 healthy adult volunteers received a dose of diclofenac suspension, a soluble tablet, or an enteric-coated tablet after suitable washout periods between doses. Plasma assays for diclofenac were drawn pre-dose and at the following time points: 0.25, 0.5, 0.75, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 6, 9 and 12 hours. This diclofenac suspension pharmacokinetic data will be available for analysis, allowing comparisons to be made between adult and paediatric patients.

In order to compare adult and paediatric pharmacokinetics, one approach could be to recruit healthy children, give them a dose of diclofenac, and draw venous blood samples at the same time points, allowing statistical comparisons of pharmacokinetic parameters such as AUC. The problem with this approach is that despite there being some justification for

recruiting healthy child volunteers for clinical drug studies (Koren G et al, 2003), dosing a child with an off-label medicine, inserting a venous cannula and withdrawing multiple blood samples is fairly traumatic and unlikely to meet ethical approval, or recruit many parents willing to enter their children.

To obtain pharmacokinetic data from paediatric subjects, the most pragmatic approach would therefore be to conduct a study in patients who are already receiving diclofenac for acute pain. The peri-operative period would seem ideal for this purpose, as not only do paediatric patients routinely receive diclofenac (Turner S et al, 1998, Conroy S & Peden V, 2001), but a venous cannula is routinely inserted which would facilitate blood sampling and, if the dose is given pre-operatively, at least some of the blood samples can be drawn in the operating theatre whilst the child is anaesthetised. The best group of patients for this purpose would be those coming for minor day-case surgery. Pre-operative analgesia is common in these patients and the more minor the procedure, the less effect surgery will have on pharmacokinetic parameters through factors such as haemodynamic fluctuations, physiological changes and metabolic stress (Kennedy JM & van Riji, 1998). This approach will be limited by clinical practice and blood sampling at pre-defined times will not be possible. It will also not be possible to collect rich pharmacokinetic data, as in the adult patients, so analysis will have to be undertaken using fewer samples per patient and possibly more patients. The differences in sampling strategies, that the paediatric patients will be undergoing surgery whereas the adult volunteers were not, and that diclofenac assays will be done in different laboratories means that taking a traditional pharmacokinetic approach will be unfeasible. Such a study, with types of patient data coming from different sources, lends itself to the population pharmacokinetic approach.

Once a final population pharmacokinetic model is reached and evaluated, it can be used as a tool to predict the pharmacokinetics in simulated patients at various doses. As the paediatric study will take place in patients undergoing surgery, valid measures of efficacy will be difficult to make. Two main factors contribute to the difficulty in collecting pharmacodynamic data to link with diclofenac pharmacokinetics in the proposed paediatric study. The first is that patients will routinely receive other analgesics outside the control of the researchers, as current clinical practice for paediatric surgery entails the use of

concomitant paracetamol, NSAIDs, opiates and local anaesthetics (Lloyd-Thomas AR, 1999, Howard RF, 2003, Morton NS, 1999). This will confound any pain scoring measures taken after the operation and in addition, blood samples will mainly be drawn in the operating theatre from anaesthetised children, making linked pain score/blood sampling impossible. Whilst it could be argued that post-operative pain scores would prove useful clinical indicators of diclofenac efficacy, the second problem is that there is currently no single validated method of scoring pain from pre-verbal to early adolescent children (Cunliffe M & Roberts SA, 2004), which is the target age range. Furthermore, collecting pharmacodynamic measures of pain will require other factors such as operation, and even arguably surgeon, to be standardised in order to improve reliability. It is therefore proposed that pharmacokinetic data be collected in any day-case surgeries, thereby increasing the pool of available patients. As mentioned earlier, clinical pharmacodynamic, pharmacokinetic and pharmacological data suggest that exposure to diclofenac as measured by AUC presents as a potentially reasonable indicator of efficacy, and that 50mg seems to be the optimum dose in adults. This study will therefore aim to predict a dose that gives a similar exposure (AUC) in paediatric patients to 50mg of diclofenac in adults.

1.5.3.2 Determining Type and Incidence of Common Diclofenac Adverse Drug Reactions

The most common cause of acute pain in children for which diclofenac is prescribed is peri-operative pain. The paediatric surgical ward is the setting where diclofenac is most commonly used off-label (Turner S et al, 1998), and so an observational study investigating the common adverse reactions in paediatric patients routinely receiving diclofenac will be undertaken. Common adverse drug reactions are considered to occur in greater or equal to one in 100 patients (Berry DC et al, 2004). In order to be 95 percent confident of seeing at least one reaction that occurs in one percent of patients, a minimum of 300 patients must be recruited:

For a reaction occurring in 1/100 patients

P(not seeing the reaction in one patient) = 0.99

P(not seeing the reaction in 300 consecutive patients) = 0.99^{300} = 0.05

Therefore there is a 95 percent chance of seeing the reaction at least once if 300 patients are studied.

To ascertain the type and approximate frequency of common adverse drug reactions of diclofenac when used for acute pain in children, an observational study design is proposed. Whilst a placebo controlled trial would be the ideal study design, allowing differences in adverse event rates between diclofenac and placebo to be judged as adverse drug reactions, the efficacy of diclofenac is already well established (Romsing J & Walther-Larsen S, 1997). Running a clinical study in which the beneficial effects of the test drug are known, thereby denying these benefits to the placebo group, would be considered unethical and it is difficult to envisage parents consenting to a trial where their children stood a chance of not receiving an effective analgesic. Modern peri-operative analgesia is multimodal with paracetamol, opioids and NSAIDs all being routinely used (Lloyd-Thomas AR, 1999). Whilst it may be possible to run a comparative study with an equally efficacious opiate (e.g. morphine), or with another NSAID (e.g. ibuprofen), this would only give information on how adverse reactions of diclofenac are different from such agents. Furthermore, to achieve successful blinding, matching formulations would need to be developed, and to ensure a good chance of seeing all of diclofenac's common adverse drug reactions, 300 patients would be required in each group.

For these reasons an observational study design will be used as it has been suggested that this method is the best way to ascertain quantitative data on adverse reactions (Kaufman DW & Shapiro S, 2000). Patients will be recruited prospectively to minimise subject bias, and information on adverse events will be collected. To determine the likelihood of an adverse event being an adverse drug reaction caused by diclofenac, causality assessment will be used. This is a similar method as that used in a recent large-scale adverse drug reaction study (Pirmohamed M et al, 2004).

1.5.3.3 Determining Type and Incidence of Rare Diclofenac Adverse Drug Reactions

To determine the incidence and type of rare adverse reactions to diclofenac for acute pain in children, much larger numbers of patients will be required than could be recruited in an observational study. As diclofenac is less commonly used in primary care for acute pain in children, and diclofenac is used for a number of indications and by several routes of administration, the use of large databases such as the UK General Practice Research Database (GPRD) for epidemiological data on adverse drug reactions would be

problematic. Firstly matching diclofenac with a diagnosis of acute pain would be required, secondly the formulation would need to be checked to ensure that the child is exposed to systemic diclofenac as opposed to topical formulations such as gel or eye drops, and thirdly the main usage of diclofenac, peri-operative pain, would not be covered.

As mentioned earlier, modern paediatric analgesia takes a multimodal approach, with diclofenac often employed alongside other analgesics. Published studies that may not necessarily use diclofenac as the test drug, may report the use of diclofenac as an adjuvant agent. An example of this is a study comparing morphine and codeine with relation to CYP2D6 genotype in 98 children, each of whom were given diclofenac 1mg/kg (Williams DG et al, 2003). Here 98 children have been exposed to diclofenac in a clinical trial setting. Whilst little can be gleaned on minor adverse drug reactions from such a study, if a patient had a diclofenac-specific rare adverse event such as allergic-type reaction, bronchospasm with nasal polyps, or acute renal failure, it would be expected to appear in the published report. It is therefore proposed that a systematic literature review, including all study designs and types, where paediatric patients have received diclofenac, may provide information on the prevalence of the rare serious adverse drug reactions of diclofenac. The systematic review will also search for case reports where diclofenac has been attributed to adverse reaction in a paediatric patient treated for acute pain, and a summary of spontaneous reporting system findings of diclofenac adverse reactions will be made (Aronson JK, 2005). This type of analysis by systematic literature review will provide quantitative and qualitative data on rare adverse drug reactions to diclofenac.

1.5.3.4 Investigating the Ontogeny of CYP2C9 using Diclofenac:4'-Hydroxydiclofenac Ratio

No clinical studies have yet sought to confirm the finding that CYP2C9 expression is adult equivalent by five months of age (Koukouritaki SB et al, 2004). Diclofenac is metabolised to 4'-hydroxydiclofenac by CYP2C9 and so could be used as an *in vivo* probe to investigate CYP2C9 expression. It is therefore proposed that samples taken for the pharmacokinetic study will also be assayed for 4'-hydroxydiclofenac and CYP2C9 genotype. Simultaneous compartmental population pharmacokinetic modelling of both diclofenac and 4'-hydroxydiclofenac will be attempted. An analysis of the relationship between age and the

formation of 4'-hydroxydiclofenac will then give an indication as to whether CYP2C9 expression appears to be mature in early infancy as the previous *in vitro* study suggests.

A potential confounding factor in this study will be CYP2C9 genotype. Any coincidental relationship between age and poor-metaboliser genotypes may risk this study drawing false conclusions. Whilst it will not be possible to look at all known SNPs affecting CYP2C9 coding regions, a broader range of alleles will be searched for than in previous studies. It would be possible to sequence the exons in the CYP2C9 gene and screen for all SNPs although this would be both costly and laborious. The Department of Forensic Pathology in Helsinki, Finland will implement a more economic, high-throughput approach using SNaPshot™ (Applied Biosystems). This technique involves using polymerase chain reaction (PCR) to replicate parts of the gene where known SNPs exist, followed by annealing of a pre-defined oligonucleotide primer complimentary to the sequence immediately prior to the potential SNP base, and adding a fluorescently labelled dideoxynucleotide triphosphate. This can then be identified using capillary electrophoresis linked to a detector (Sistonen J et al, 2005). Using this method will allow the detection of multiple alleles in a single economic step.

Chapter TWO: Pharmacokinetics of Diclofenac in Children

2.1 Introduction

The term pharmacokinetics can be simply defined as: "a mathematical description of drug passage through the body" and is the study of the fate of drugs after administration encompassing absorption, distribution, metabolism and elimination. The term pharmacodynamics is simply defined as: "what the drug does to the body" and is concerned with the biochemical, physiological and overall clinical effect of a drug. The term pharmacokinetics was probably first used in the 1950s although recognition of the link between dose, blood concentration and effect was discussed in relation to ether-based anaesthesia in 1847 (Wagner JG, 1981). Pharmacokinetic data derived from measuring drug concentrations in the body (usually through blood sampling) is often analysed empirically using compartmental modelling, with passage between usually one or two compartments being explained by summing exponential terms relating drug concentration and time (Ronfeld RA & Benet LZ, 1977).

It has long been recognised that such empirical compartmental modelling, whilst often providing a good description of pharmacokinetic data, may cause over-simplification of the complex process of absorption, distribution, metabolism and elimination of drugs (Riegelman S et al, 1968). Multiple compartment modelling using realistic volumes for body fluids and organs were described as early as 1937 (Wagner JG, 1981). Such physiologically-based systems have been extensively used to describe pharmacokinetic data, computer technology enabling whole-body models to be developed. The usual application of such models is for predicting pharmacokinetics in man using data on the drug's physico-chemical properties, *in vitro* biochemistry, and knowledge from animal pharmacokinetic studies; recently physiologically-based models have been described that aim to predict paediatric pharmacokinetics (Edgington AN et al, 2006, Johnson TN et al, 2006). Pharmacokinetics is now broadly split into two fields: whole-body physiologically-based modelling usually used in early-phase drug development for predicting initial doses, and empirical compartmental modelling often with a population approach, to describe drug handling, and generally used in the later stages of drug development and clinical practice.

Increasing overlap between these two fields is now occurring with the recognition that empirical models must be more physiologically plausible (Aarons L, 2005).
This study will use empirical compartmental modelling of population pharmacokinetic data. Population pharmacokinetics was pioneered in the 1970s with the recognition that traditional intensive blood sampling methods could not be used routinely in the clinical setting where multiple, standardised blood sampling is often unfeasible. However, pharmacokinetic data collected in such a setting, for example during therapeutic drug monitoring, could potentially yield useful information on a drug's pharmacokinetics in a relevant population (Sheiner LB et al, 1977). By the early 1990s the theory and application of population pharmacokinetics had become recognised as a potentially useful tool in drug development (Aarons L, 1991, Sheiner LB & Ludden TM, 1992) and presently it forms the main method of summarising pharmacokinetic data generated in phase II and III of the drug development process (Bonate P, 2005).

The population pharmacokinetic approach does not require a complete pharmacokinetic profile for each individual, meaning that fewer blood samples per patient are required than with traditional intensive sampling methods. In the traditional pharmacokinetic approach, an individual model may be written as shown in equation 2.1:

$$y_j = f_j(\theta_j, \chi_j) + \varepsilon_j \qquad \text{Equation 2.1}$$

Where:

y_j = a measured value (e.g. plasma drug concentration).
θ_j = pharmacokinetic parameters (e.g. CL, V_D, Ka).
χ_j = measured quantities (e.g. dose, sampling time).
f_j = a function relating θ_j and χ_j.
ε_j = measurement of error.

Whereas the population model may be summarised as in equation 2.2:

$$y_{ij} = f_{ij}(\theta_j, \chi_{ij}) + \varepsilon_{ij} \qquad \text{Equation 2.2}$$

Where:

y_{ij} = for individuals 1.....,n_j concentrations measured at doses and times χ_{ij}.
θ_j = pharmacokinetic parameters of the population (e.g. CL, V_D, Ka).
f_{ij} = a function predicting y_{ij} from θ_j and χ_{ij}.
ε_{ij} = measurement of error.

To model population pharmacokinetic data, the simplest approach is the two-stage method whereby subject parameters (θ) are estimated individually, and population parameter and error estimates made in the second step (Ette EI & Williams PJ, 2004a). The problem with the two-stage approach in the proposed study is that sparse data (three samples per dose) will be obtained from the paediatric patients, making it unlikely that all pharmacokinetic parameters can be estimated for each individual. Even with rich data, using the two-stage approach allows unbiased estimates of population parameter values, but estimates of variability tend to be biased towards higher values (Ette EI & Williams PJ, 2004a). An approach that obtains estimates of population pharmacokinetic parameters and their variability in a single step is therefore required. This can be achieved with computer programs that implement non-linear mixed effects modelling such as NONMEM (*NONMEM User Guides*, 2006).

The term mixed effects modelling refers to the possibility of deriving estimates for the pharmacokinetic parameters, or fixed effects, and a measure of their variability, or random effects. The random effects can be further subdivided into interindividual and intraindividual variability on each parameter estimate (η), and quantification of the residual variability (ε). The population model in NONMEM is therefore:

$$y_{ij} = f_{ij}(\theta_j, \chi_{ij}, \eta_i) + \varepsilon_{ij} \qquad \text{Equation 2.3}$$

Where:

η_i = inter and intraindividual variability.
ε_{ij} = residual variability.

A NONMEM run seeks to derive estimates for the fixed effects and random effects by minimising an objective function value. An example of a simple objective function value estimation would be:

$$OFV(\theta^{\wedge},y) = \sum_{i=1}^{n} [y_i - f(\theta^{\wedge}, \chi_i)]^2 \qquad \text{Equation 2.4}$$

Where:

n = number of individuals (i=1 denotes a single observation per individual).
$\theta^{\wedge}$ = a vector of θ, the population parameter value.
$OFV(\theta^{\wedge},y)$ = the objective function value derived from the population pharmacokinetic estimates ($\theta^{\wedge}$) and observations (y).

Least squares regression then finds the minimum value of the objective function, that is to say that values for $f(\theta^{\wedge}, \chi_i)$ are found which are as close as possible to observed measurements (y_i). The objective function value derived in NONMEM is slightly more complicated as it accounts for the random effects where variability can change with observations. NONMEM uses weighted least squares to minimise the objective function (Bonate PL, 2005) as shown in equation 2.5:

$$OFV(\theta^{\wedge},y,\varepsilon^{\wedge}) = \sum_{i=1}^{n} \{[1/g(\theta^{\wedge},\chi_i,\varepsilon^{\wedge})] \times [y_i - f(\theta^{\wedge}, \chi_i)]^2 + Ln[g(\theta^{\wedge},\chi_i,\varepsilon^{\wedge})]\}$$

Equation 2.5

Where:

$g(\theta^{\wedge},\chi_i,\varepsilon^{\wedge})$ is a model of the variability of the observations (weighting function)
$Ln[g(\theta^{\wedge},\chi_i,\varepsilon^{\wedge})]$ is a penalty term to stop $g(\theta^{\wedge},\chi_i,\varepsilon^{\wedge}) \rightarrow \infty$ and therefore $OFV \rightarrow 0$.

NONMEM uses a first-order Taylor series expansion where terms are summed from a function calculated from the derivative at each sampling time. The random effects are linearised in NONMEM and assumed to have a mean of zero; this is known as the first-order approximation method and is widely used for population pharmacokinetic analyses (Bonate PL, 2005, Ette EI & Williams PJ, 2004b). NONMEM also has a first-order conditional estimation method which linearises the random effects around the individual parameter estimate (as opposed to the population estimate in the first-order method) and the 'interaction' option specifies that residual error is calculated on the individual parameter estimate rather than population estimate (*NONMEM User Guides*, 2006).

The process of model building in population pharmacokinetics has three major components: defining the structural model, which usually consists of a number of compartments with drug transfer between them modelled by zero or first-order rates; the

statistical model, which estimates interindividual and intraindividual variability on the structural parameter estimates, and estimates the residual variability; and the covariate model which investigates how demographic or other factors influence parameter estimates. The challenge of model building is to find a parsimonious model that describes observed data well. Once a satisfactory model is reached, it is then possible to make predictions on the pharmacokinetics, and therefore dose, for simulated patients (Ette EI et al, 2004c).

Although empirical compartmental modelling will be used in this study, two factors will be introduced in the model building process to help ensure parameter estimates are physiologically plausible. The first is the common practice of forcing the random effects assigned to CL and V_D to covary, in recognition that it would be expected for these parameters to increase together (a larger individual is likely to have a higher V_D and higher CL than the population value). The second factor will be to use allometric scaling on the fixed effects, a detailed discussion of which was provided in Chapter One.

Diclofenac inhibits COX-2 in a time-dependent manner *in vitro* (Blobaum AL & Marnett LJ, 2007, Rowlinson SW et al, 2003) and 50mg appears to be the optimum dose for acute pain in adults (McQuay HJ & Moore RA, 1998). As diclofenac inhibits COX-2 even at nanomolar concentrations (Hinz B et al, 2003), then effect is probably better correlated with drug exposure (AUC) rather than a target tissue concentration. If it is assumed that COX-2 expression at sites of tissue injury does not differ between children and adults (no references were found to support or refute this assumption), then a paediatric dose that produces a similar AUC to 50mg in adults should in theory give a similar therapeutic effect; thus AUC will be used as a surrogate marker for efficacy in this study.

2.2 Aims

To describe the pharmacokinetics of diclofenac when used for acute pain in children.
To recommend a suitable dose of diclofenac for acute pain in children that gives a similar effect to 50mg in adults.

2.3 Objectives

To collect blood samples for diclofenac analysis in children receiving diclofenac suspension during minor surgery.
To develop and evaluate a population pharmacokinetic model with the paediatric data pooled with adult pharmacokinetic data provided by the suspension manufacturer.
To recommend a paediatric dose based on simulations from the final pharmacokinetic model.

2.4 Methods

2.4.1 Recruitment

After approval from an independent ethics committee (Appendix 7.1), parents of patients scheduled for day surgery at Great Ormond Street Hospital for Children were telephoned in the week prior to admission to inform them about the study and field any questions. On admission to hospital, parents and patients were provided with an information sheet (Appendices 7.2 & 7.3) and the opportunity to seek clarification about any aspect of the study from the researcher. Written informed consent from parents, and where appropriate assent from patients was obtained, study inclusion criteria being:

1. Patients aged one to 12 years.
2. Scheduled to undergo minor day-case surgery.
3. Written informed parental consent, and patient assent where appropriate.

The exclusion criteria were:

1. Diclofenac or other NSAID allergy.
2. History of hepatic disease, renal dysfunction, known coagulation defects, gastrointestinal bleeding.
3. One or more doses of diclofenac within the previous 24 hours.

On admission to the day surgery ward, as part of the patient's routine medical examination in preparation for surgery, the following observations were made: weight, height, temperature, pulse rate, respiratory rate, blood pressure and blood oxygen saturation. Past medical history and drug history was also recorded, along with full details of any drugs (including dose and frequency) taken in the previous week. All relevant demographic and medical information was recorded on a case report form (Appendix 7.4); patients were given a unique code number by which the case report form was made anonymous.

For logistic reasons (one investigator and the need to observe dosing and blood sampling in addition to delivering blood samples to the laboratories) a maximum of two patients per surgical list could be recruited. At the start of recruitment for this study, diclofenac suppositories were granted a license for acute pain in children aged six years and over (*Diclofenac SPC*, 2005) and so although the inclusion criteria encompassed children up to 12 years, it was decided to preferentially recruit patients under six years, as this was the age group for which diclofenac remained unlicensed.

Once recruited a bottle of diclofenac sodium 10mg/mL (Rosemont Pharmaceuticals Ltd., UK) was dispensed from the hospital pharmacy and a digital watch (Constant CT011 quartz sport LCD watch) allocated to the patient and clipped to the medical notes. A single oral dose of diclofenac 1mg/kg was prescribed and administered prior to surgery. At the discretion of the anaesthetist, some patients were also given midazolam 0.5mg/kg (Special Products Ltd., UK) and/or paracetamol 20mg/kg (Pfizer Consumer Healthcare Ltd., UK) prior to surgery. Demographic and medication details were recorded on a case report form (Appendix 7.4).

2.4.2 Diclofenac Administration and Blood Sampling

As a requirement for undergoing surgery, children had undergone an overnight fast. Diclofenac doses were rounded to the nearest 5mg (0.5mL) and measured by oral syringe (Baxa Ltd., UK); the volume checked by a second person. Patients were observed when taking the dose and time to the nearest minute recorded using their digital watch. Any patient who did not appear to swallow the whole dose was withdrawn from the study.

The first blood sample was obtained in the anaesthetic room. Anaesthesia was induced with either inhalational sevofluorane (Baxter Ltd., UK) or intravenous propofol 2-4mg/kg (Fresenius Kabi Ltd., UK) depending on the child's age and parental/patient preference. In the case of inhalational induction, once the patient was unconscious an intravenous cannula was inserted into a peripheral vein and the first blood sample was drawn. Where patients had intravenous anaesthesia, a blood sample was drawn before the propofol was administered if the patient was calm following cannulation, otherwise patients were anaesthetised and then a blood sample drawn following the removal of at least 0.5mL of dead space blood. The removal of dead space blood prior to sampling aimed to discard the contents of the cannula tip, which may not be thoroughly mixed with circulating venous blood.

At the end of the surgical procedure a further blood sample was collected, and on the day surgery ward during removal of the venous cannula prior to discharge, a third sample was collected. On both of these occasions at least 0.5mL of dead space blood was removed prior to sampling. The aim was to obtain three blood samples per dose, two of which could often be taken when the child was anaesthetised, at times that were most convenient during the peri-operative period. Where a blood sample could not be obtained, for example if the patient pulled out their venous cannula or no blood could be withdrawn, no provision was made to re-insert the cannula. These patients with less than three samples per dose were still included in the population pharmacokinetic analysis.

In each case, the time of blood sampling was recorded to the nearest minute using the patient's digital watch. For the diclofenac assay 0.5-1mL of whole clotted blood was collected and delivered to the Department of Chemical Pathology at Great Ormond Street Hospital where they were centrifuged, serum extracted and frozen at -20°C. An additional 1mL of whole blood was also collected at the first sampling point; this sample was frozen for CYP2C9 genotyping (Chapter Five). Once the final blood sample had been drawn, the digital watch was presented to the child as a gift for taking part in the study.

2.4.3 Diclofenac Assay

The analytical unit at St George's Hospital, London, undertook diclofenac assays. Serum was extracted from clotted whole blood samples and the assay method used was sequential high performance liquid chromatography followed by mass spectrometer detection (HPLC/MS). Solid phase extraction was used to clean the samples prior to HPLC using an Altima C18 column with methanol and ammonium acetate (5mmol/L) 50:50 (Rathburn Chemicals Ltd., UK) used as the mobile phase. Ketoprofen (Sigma-Aldrich Ltd., UK) was used as internal standard and detection was performed by an AP1400 mass spectrometer with nitrogen as the collision gas. Diclofenac sodium (Sigma-Aldrich Ltd., UK) was used for calibration. Output was analysed using Analyst (version 1.3.2) software that performed integration of diclofenac detection peaks. The lower limit of detection for diclofenac was 10.1ng/mL. The intra-assay precision, defined by the percentage coefficient of variation, ranged from 0.81 to 11.78 percent, the mean percentage accuracy ranged from 94.16 to 112.59 percent.

2.4.4 Adverse Event Monitoring

Adverse events were monitored for and recorded throughout the hospital admission. In addition, parents were telephoned approximately one week following discharge to check for any delayed adverse events. Further detail on adverse event monitoring is given in Chapter Three.

2.4.5 Adult Pharmacokinetic Data

The sponsor of this study provided pharmacokinetic data on diclofenac suspension in adult volunteers collected during a bioequivalence study undertaken by the Shandon Clinic Ltd., Cork, Ireland. After fasting overnight the volunteers received a single 50mg dose of diclofenac suspension (Rosemont Pharmaceuticals Ltd., UK) and 6mL blood samples for diclofenac assay were drawn at: 0.25, 0.50, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 9.0 and 12.0 hours post-dose. Where actual sampling times deviated from this schedule by more than one minute, actual time was recorded, and this was used in the population pharmacokinetic analysis. As with the paediatric samples, HPLC/MS was used although naproxen (Sigma Ltd., UK) was the internal standard and plasma rather than serum was assayed. The assay lower limit of quantification was 10ng/mL. The intra-assay precision,

defined by the percentage coefficient of variation, ranged from 5.54 to 12.09 percent, the mean percentage accuracy ranged from 94.68 to 104.09 percent.

2.4.6 Pharmacokinetic Model Building Strategy

Raw plots of serum (paediatric) or plasma (adult) diclofenac concentration versus time were generated in Excel (Microsoft Office 2003) and inspected for possible structural models. Structural modelling decisions were largely based on the rich adult data. Approximate initial parameter estimates for fixed effects were derived from the raw plots by estimating AUC and slopes of the absorption and elimination phases. Assay reports of diclofenac concentrations were given in ng/mL and diclofenac was dosed in mg of diclofenac sodium. For this reason, all mass units were changed to nanomoles (assuming a molecular weight of 318.13g for diclofenac sodium and 296.15g for diclofenac) and all volume quantities were transformed to litres. This information from both the adult volunteers and paediatric patients was pooled together and entered into a NONMEM-format data file.

Pharmacokinetic model building was undertaken using a Dell D600 Notebook with Intel Pentium processor (2.00GHz) running NONMEM (version 6.0) compiled with a Compaq Visual Fortran (version 6.1) compiler. The estimation method chosen in NONMEM was first-order conditional estimation with interaction. Allometric scaling was added to all clearance and volume fixed effects *a priori* and standardised to a body weight of 70kg (Meibohm B et al, 2005) according to the following relationships:

$$\text{Clearance} = \theta_{CL}(w/70)^{0.75} \qquad \text{Equation 2.6}$$

$$\text{Volume} = \theta_{V}(w/70) \qquad \text{Equation 2.7}$$

Where:

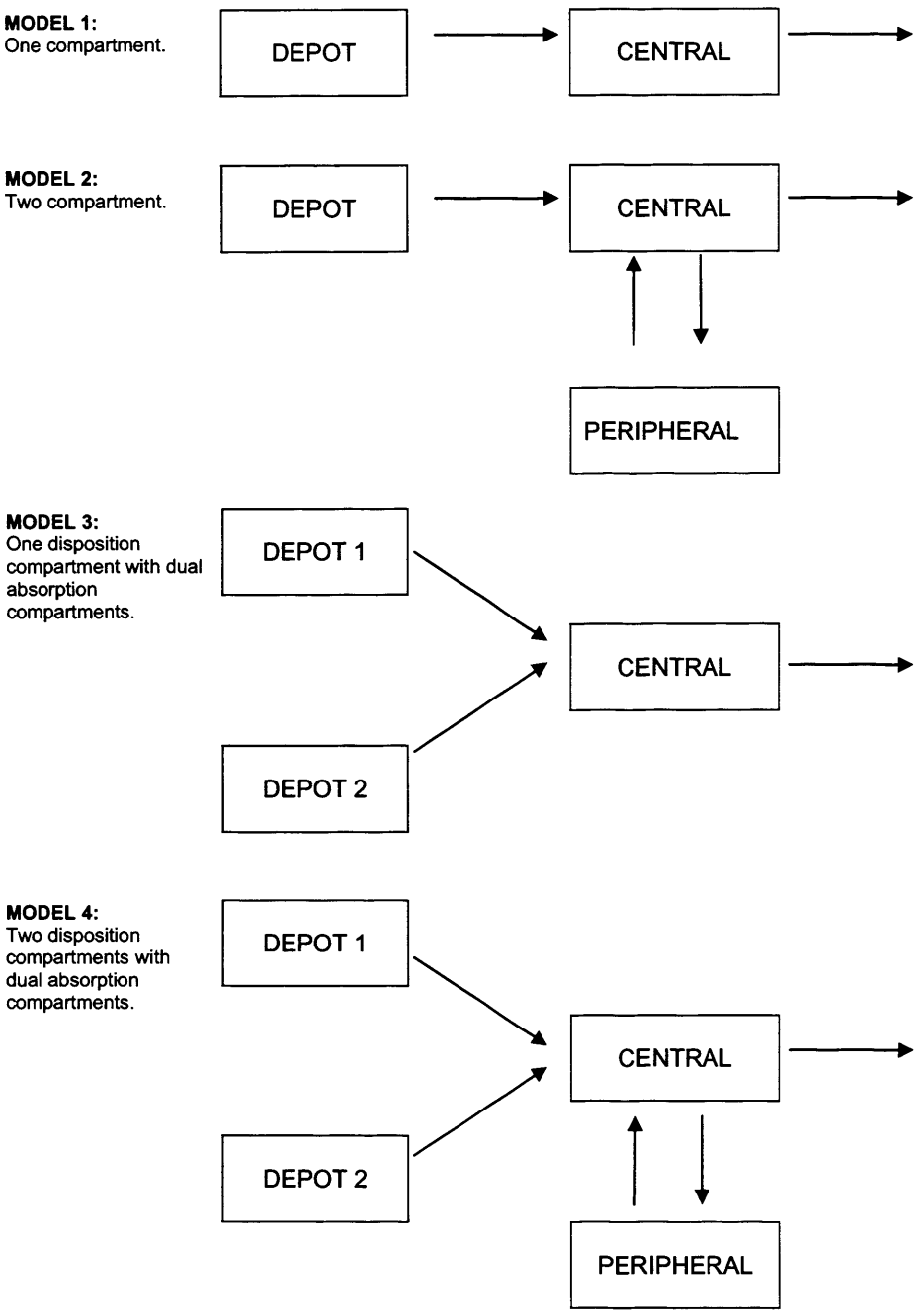
θ_{CL} = Population estimate of clearance term (L/hr).
θ_{V} = Population estimate of distribution volume term (L).
w = Body weight (kg).

Inter-individual variability (η) was assumed to be log-normally distributed (so negative parameter values could not be estimated), and a block covariance matrix was used to force clearance and volume terms to co-vary. As diclofenac assays were performed in different

laboratories for the adult and paediatric data, and the adult samples were plasma whereas the paediatric samples were serum, estimation of residual variability was undertaken separately for the two groups, with proportional, additive and mixed proportional-additive error models tested.

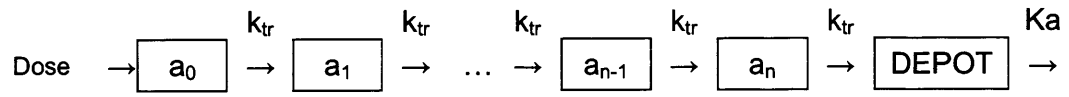
The model building process started with estimating population parameters with only residual variability. Inter-individual variability was added in a stepwise fashion, firstly to clearance and volume parameters, and then to absorption parameters. When the final structural model was stabilised (successful run in NONMEM with realistic parameter estimates and reasonable goodness-of-fit plots) between occasion variability (Karlsson MO & Sheiner LB, 1993) was added to clearance and volume terms. Graphical analysis of final parameter estimates for clearance and volume versus covariates age, height, sex and ethnicity were examined for trends not explained by the allometric size models. The final model was then evaluated to assess its predictive performance.

Figure 2.1 gives a schematic representation of the structural models investigated. Absorption between depot and central compartments was investigated by zero, first, mixed zero-first order rates and by the transit compartment model. The transit absorption model replaces the lag time estimated in traditional pharmacokinetic models and consists of a number of transit compartments leading to the depot compartment. A schematic diagram of the transit absorption model is given in figure 2.2.



Arrows represent drug transfer between compartments. Elimination from the central compartment and transfer between central and peripheral compartments was modelled by first-order rates. Absorption from the depot to the central compartments was modelled by zero, first, mixed zero-first rates and transit absorption models.

Figure 2.1: Schematic diagram of structural models investigated.



a_0 = Amount of drug in compartment 0.
k_{tr} = Rate constant of drug transit between compartments.
Ka = Rate constant of drug transit into the central compartment.

Figure 2.2: Schematic diagram of transit absorption model.
Adapted from: Savic RM et al, 2007.

The amount of drug in the absorption compartment time t is a product of the amount entering it, and the amount leaving it. This can be expressed by the following equation (Savic RM et al, 2007):

$$\frac{\delta a_{(DEPOT)}}{\delta t} = \underbrace{\left(Dose \cdot \frac{(k_{tr} \cdot t)^n \cdot e^{-ktr.t}}{n!} \cdot k_{tr} \right)}_{\text{Relates to drug transfer through transit compartments into depot compartment.}} - \underbrace{\left(Ka \cdot a_{(DEPOT)} \right)}_{\text{Relates to drug transfer into central compartment.}} \qquad \text{Equation 2.8}$$

Where:
$a_{(DEPOT)}$ = Amount of drug in the DEPOT compartment.
t = Time.
n = Number of transit compartments.
n! = Stirling's approximation.

In NONMEM code, the time for drug to reach the depot compartment is called the mean transit time (MTT) and effectively replaces the conventional lag time. It is given by the expression:

$$MTT = \frac{n + 1}{k_{tr}} \qquad \text{Equation 2.9}$$

Using the relationships in equations 2.8 and 2.9, MTT, n and Ka were all estimated as fixed effects in NONMEM.

In the case of the dual absorption compartment models, a full dose was administered to both compartments. Bioavailability from each compartment was estimated in NONMEM as a fixed effect, with limits forcing the combined bioavailability to equal 100 percent, thereby only allowing one whole dose into the central compartment. As some paediatric patients received two doses on separate occasions, between occasion variability was estimated for V_D and CL.

2.4.7 Pharmacokinetic Model Evaluation

NONMEM table files were used to generate diagnostic plots in Excel and Xpose (version 4.0 run in R version 2.4.0) for general structural model and residual error model evaluation during the model building process. Due to the risk of random effect shrinkage (Karlsson MO & Savic RM, 2007) with the sparse paediatric data, separate diagnostic plots of individual prediction versus observation for the adult and paediatric data portions were produced to compare with the pooled plots.

The main form of final model evaluation was performed with the following simulation-based techniques: visual predictive check, mirror plots with Xpose and comparison of calculated AUC from the raw data and model-derived simulations (posterior predictive check).

The visual predictive check entailed simulating new data based on distributions derived from the final parameter estimates. A new data structure was constructed, with 100 patients with identical demographic details to the original dataset but sampling times at 0.5, 1, 2, 3, 5 and 12 hours so that a median value at each time point could be derived. Using NONMEM, 100 new datasets based on this data structure were simulated, and the median, fifth and 95th percentiles plotted on top of the original data. This plot gives a visual measure of model performance and ideally around five percent of the original data should fall above and below the fifth and 95th percentiles of the model-simulated data.

Xpose was also used to produce mirror plots from datasets simulated from the final model. Plots derived from the original data compared with plots from three randomly selected simulated datasets were compared visually.

The final predictive check was to compare $AUC_{(0\text{-}12h)}$ calculated from the raw adult data using non-compartmental analysis (model 200, extravascular input with plasma/serum concentrations) in WinNonlin (version 3.2, Pharsight Ltd.) with $AUC_{(0\text{-}12h)}$ values derived from model simulations. The terminal elimination rate constant was derived from the final three data points of the elimination phase. This tested how well the model was able to predict $AUC_{(0\text{-}12h)}$, a key parameter used as a surrogate for efficacy in this study.

2.4.8 Simulations

Once the final model and population parameter values had been derived, simulated doses of 0.5, 1, 1.5 and 2mg/kg were created with NONMEM. A new data file using the demographic information from the original 100 patients was created, with blood sampling times at 0.5, 1, 2, 3, 5 and 12 hours to allow for $AUC_{(0\text{-}12h)}$ calculation in each age group. For each dose level, 100 simulated datasets were created, from which median $AUC_{(0\text{-}12h)}$ values for the age ranges 1 to 3 years, 4 to 6 years and 7 to 12 years were calculated. The $AUC_{(0\text{-}12h)}$ for 50mg in adults was also calculated; paediatric $AUC_{(0\text{-}12h)}$ values at each dose level were divided by this adult 50mg $AUC_{(0\text{-}12h)}$ giving a ratio. The closest value of this ratio to one gave the most similar exposure and so was the recommended dose.

2.5 Results

2.5.1 Recruitment

Over a ten-month period 96 patients were approached and 74 provided informed consent giving a recruitment rate of 77 percent. Reasons for not consenting were not systematically recorded as a statutory requirement of the ethics committee was that parents/patients were allowed to withdraw without giving a reason. A schematic diagram of patient recruitment is given in figure 2.3.

One patient's operation was cancelled as she ate a chocolate biscuit procured from her mother's unattended handbag during the nil-by-mouth period prior to surgery. This patient was readmitted within the recruitment phase, underwent surgery and so became one of the 70 included patients. The patient who took the dose but did not undergo surgery became distressed in the anaesthetic room and refused to be anaesthetised. As the procedure was elective laser treatment for removal of a birth mark, it was decided not to restrain the child.

This patient had received a full dose of diclofenac so was followed-up for adverse events, the results of which are reported in Chapter Three.

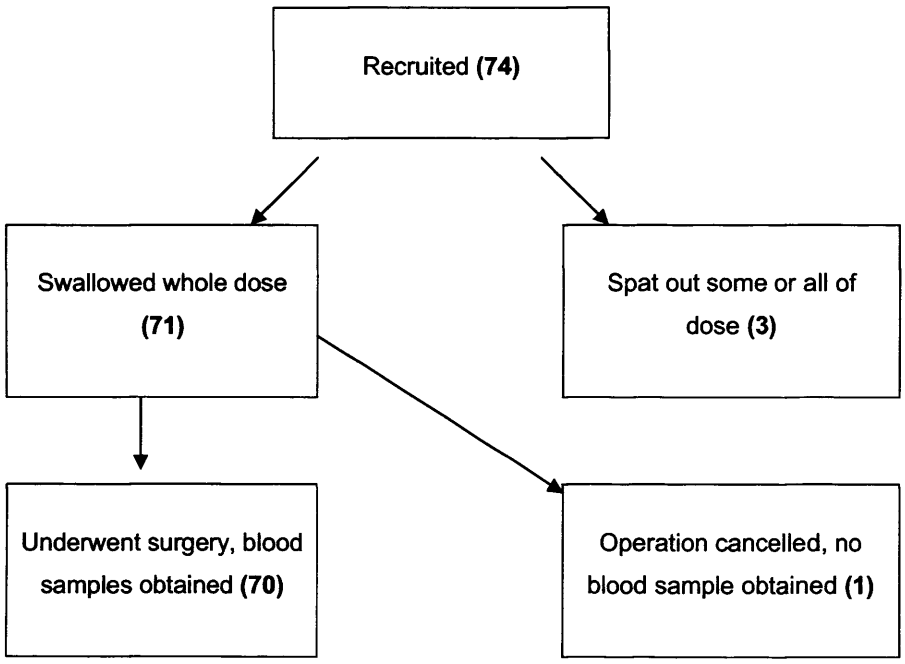


Figure 2.3: Paediatric patient recruitment for the pharmacokinetic study.

2.5.2 Demographics

Demographic details for both the paediatric and adult patients are given in table 2.1, graphical representation is also given in figure 2.4.

Table 2.1: Demographic details of paediatric patients and adult volunteers included in the pooled population pharmacokinetic analysis.

		Frequency given as mean (range) or number (percentage) as appropriate		
		Children n=70	**Adults n=30**	**Pooled n=100**
Age (years)		3(1-12)	21 (18-28)	9(1-28)
Weight (kg)		17(9-37)	72(48-94)	34(9-94)
Height (cm)		101(69-146)	170(158-187)	122(69-187)
Male		41(59%)	14(47%)	55(55%)
Female		29(41%)	16(53%)	45(45%)
Surgery type:				
	Dermatology	54(77%)	-	-
*	General	12(17%)	-	-
*	Plastic	4(6%)	-	-

* Excision of lesions undertaken by general and plastic surgeons, classification made by surgeon specialty.

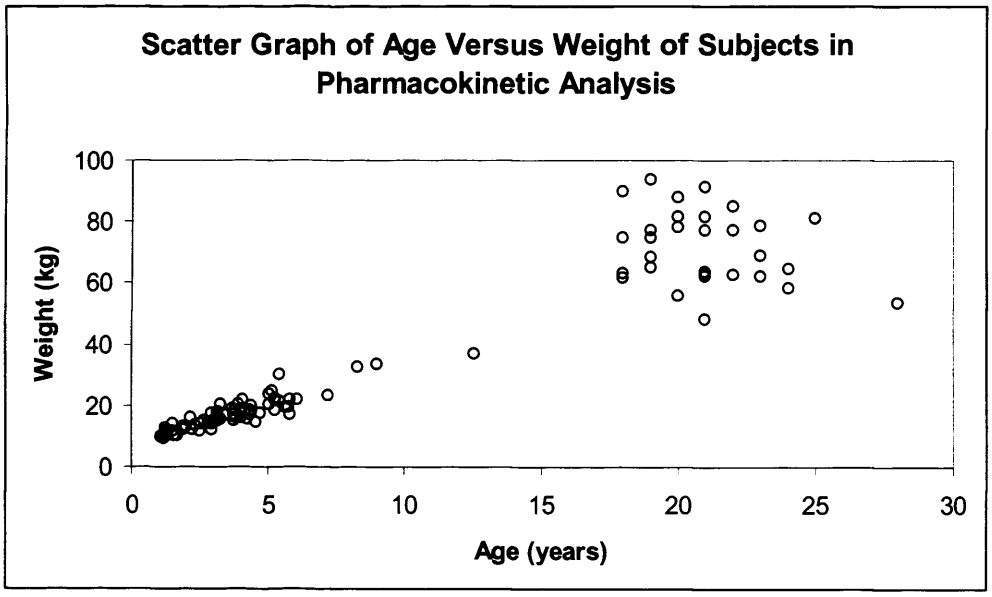


Figure 2.4: Comparison of age and weight in subjects included in the pooled diclofenac pharmacokinetic analysis.

The dermatology patients all underwent laser removal of port-wine stain, the general surgery patients underwent hernia repair, orchidopexy or lesion excision, the plastic surgery patients all underwent lesion excision. The majority of patients underwent laser surgery mainly because there was at least one list per week (two on alternate weeks) most of which contained at least two potentially eligible patients.

2.5.3 Raw Data

A total of 558 (206 paediatric, 352 adult) diclofenac concentrations were used in the pooled pharmacokinetic analysis (raw data is provided on a CD located in the back cover of this thesis). Sampling times for the paediatric patients ranged from 0.2 to 6.47 hours post dosing, with at least two samples per dose being drawn from all but one patient, where only one sample was obtained. Reasons for not obtaining the full three samples were: eight parents refused to allow the third sample (when the child would be awake); no blood could be withdrawn from the cannula; on three occasions the patient pulled out the cannula in the recovery area. No provision was made to re-insert venous cannulas as the population pharmacokinetic approach does not require full profiles from individual patients and so the added distress caused to the patient is unjustified. Seven patients (all dermatology laser surgery) were readmitted during the recruitment period for surgery and so samples on two occasions were taken. The total number of blood samples from any single paediatric patient was seven (six for diclofenac assay during two doses and one for CYP2C9 genotyping). No paediatric sample was below the limit of quantification (BLQ) and the values of BLQ samples in the adult data set (mainly post six hours) were not reported by the laboratories. Plots of diclofenac concentration versus time are given in figures 2.5 and 2.6.

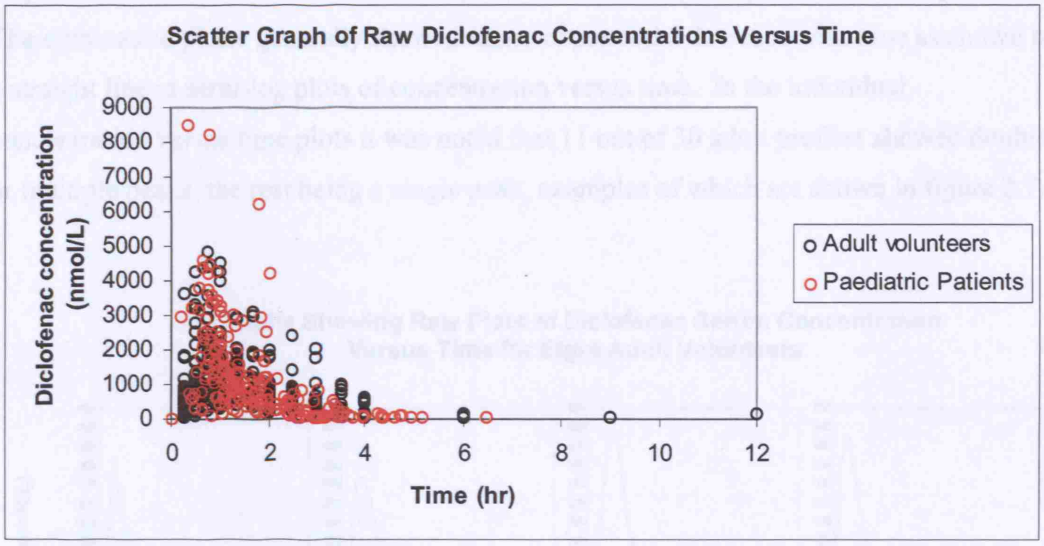


Figure 2.5: Scatter plot of raw diclofenac pharmacokinetic data.

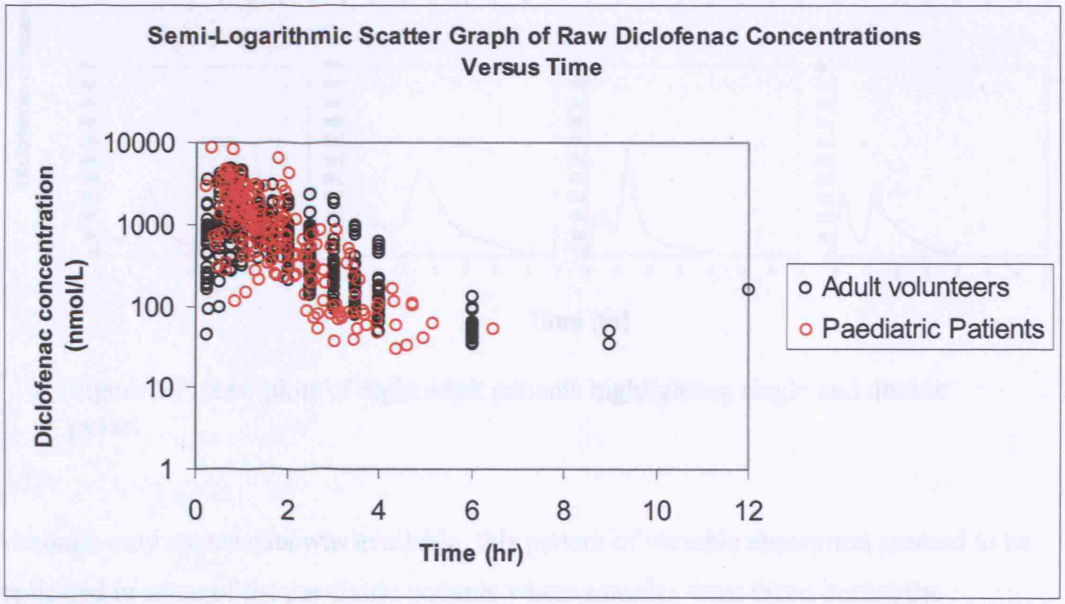


Figure 2.6: Semi-logarithmic scatter plot of raw diclofenac pharmacokinetic data.

Inspection of each individual's raw data revealed the absorption phase to be most erratic. The elimination phase generally showed mono-exponential decrease with time as shown by a straight line in semi-log plots of concentration versus time. In the individual concentration versus time plots it was noted that 11 out of 30 adult profiles showed double or multiple peaks, the rest being a single peak, examples of which are shown in figure 2.7.

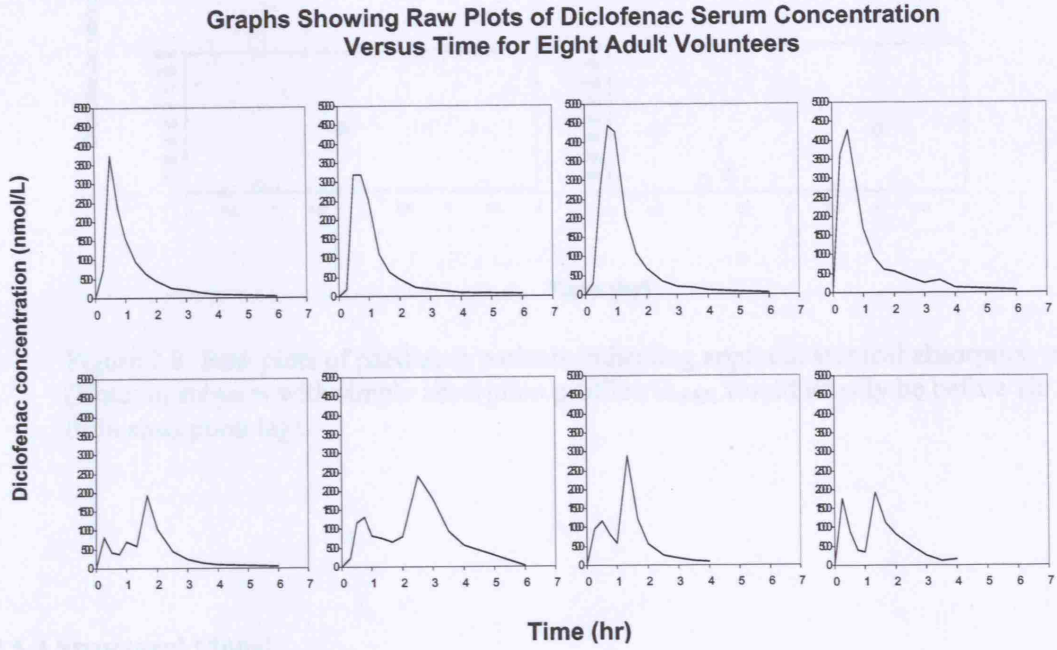


Figure 2.7: Raw plots of eight adult patients highlighting single and double peaks.

Although only sparse data was available, this pattern of variable absorption seemed to be replicated in some of the paediatric patients where samples were taken during the absorption phase, as demonstrated in figure 2.8.

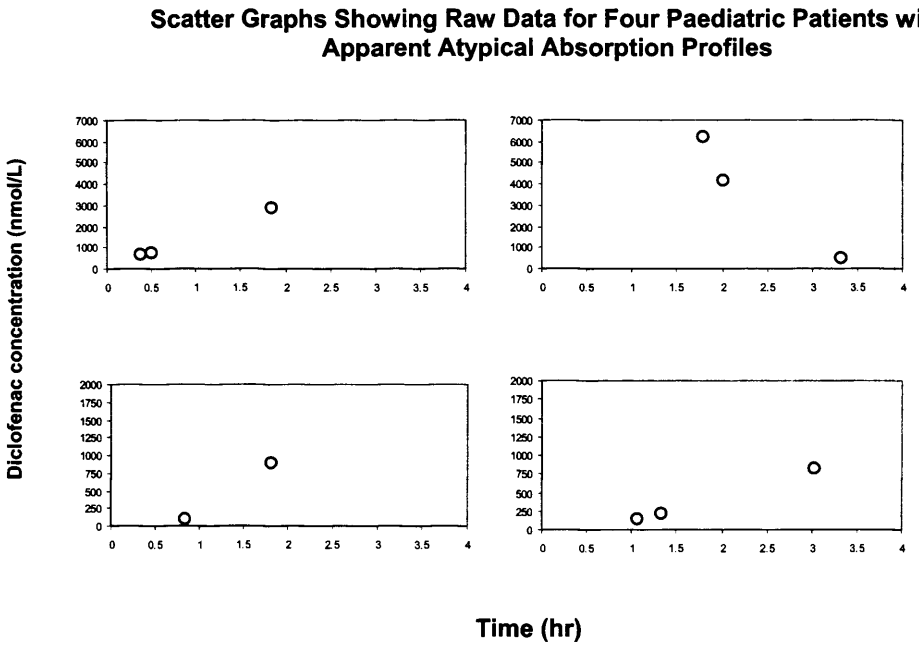


Figure 2.8: Raw plots of paediatric patients indicating apparent atypical absorption profiles. (Note: in subjects with simple absorption profiles C_{MAX} would usually be before 1hr with little absorption lag).

2.5.4 Structural Model

Simple one and two compartment models using the pre-defined algorithms in NONMEM gave poor goodness-of-fit as defined by plots of observed concentration versus individual and population predictions; the dual absorption compartment model was implemented with differential equations using ADVAN6, the general nonlinear mixed effect model in NONMEM that allows user-defined compartmental modelling with differential equations explaining drug transport between compartments. The dual absorption compartment model was clearly better than single absorption compartment models in that it was able to predict both single and double peak profiles. The terminal elimination phase looked linear on plots of Log diclofenac concentration versus time suggesting a peripheral disposition compartment was unnecessary. The basic structural model was therefore Model 3 (figure 2.1).

Refinements of this model focussed on evaluating absorption rates from the depot to central compartment. Zero, first, mixed zero-first order rates and transit absorption models were investigated with all giving similar final parameter estimates for CL and V_D; the transit absorption compartment model subjectively produced better goodness-of-fit plots and so was evaluated further. Figure 2.9 shows plots of observed diclofenac concentrations versus population model predictions and figure 2.10 shows observations versus individual (including inter/intraindividual variability) model predictions for both the pooled data and adult and paediatric data alone.

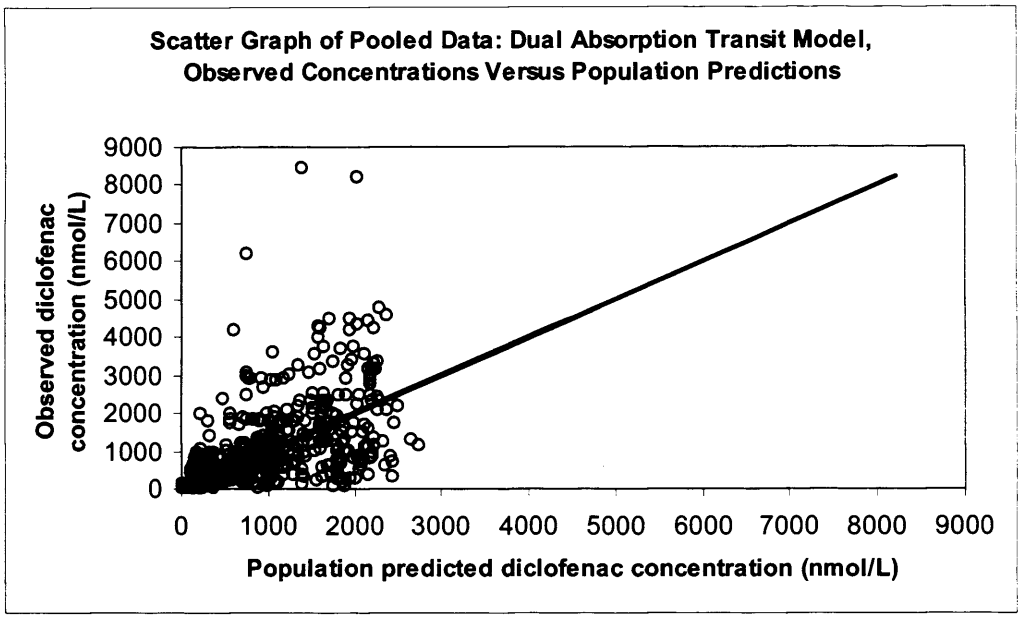
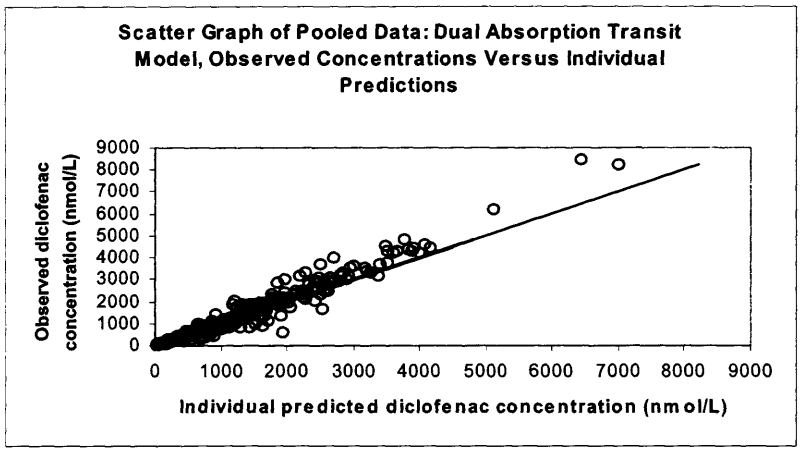
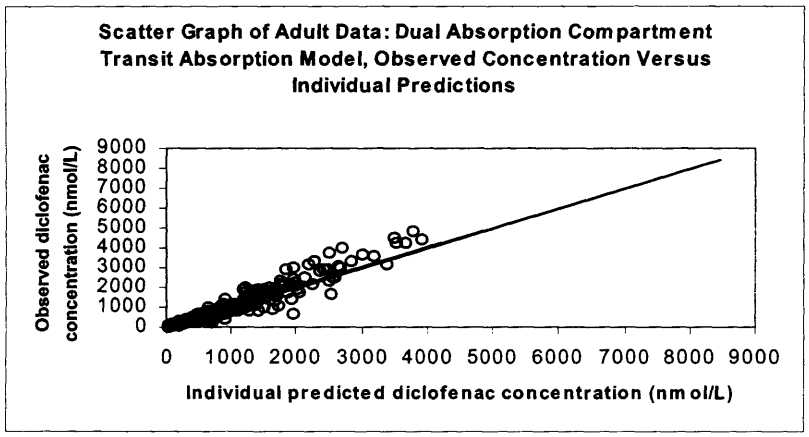


Figure 2.9: Scatter plots of observed diclofenac concentrations versus population model predictions.

a.)



b.)



c.)

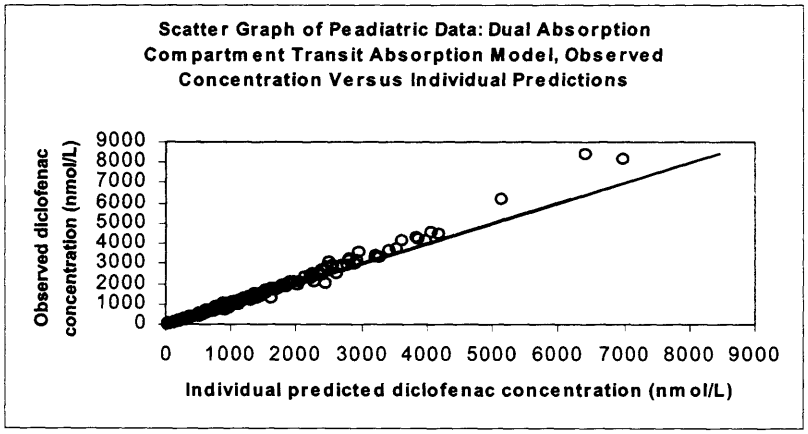


Figure 2.10: Scatter plots of observed diclofenac concentrations versus individual model predictions (highlighting shrinkage in the sparse data) for: a.) All data. b.) Adult data. c.) Paediatric data. Line of unity added for reference.

Figure 2.11 shows plots of weighted residual error (WRES) versus time. WRES is a transformation of residual error (difference between population prediction and observations) into standard deviation units. A plot of WRES versus time was used to diagnose the structural model, with a well-specified structural model showing a trend-less pattern with most points falling between +2 and –2, that is to say within two standard deviations of zero, the point at which the population prediction equals the observed concentration. This plot gives an indication of goodness-of-fit of the structural model, as large deviations from zero in a single direction would suggest the structural model is poor as it fails to explain concentrations at these time points.

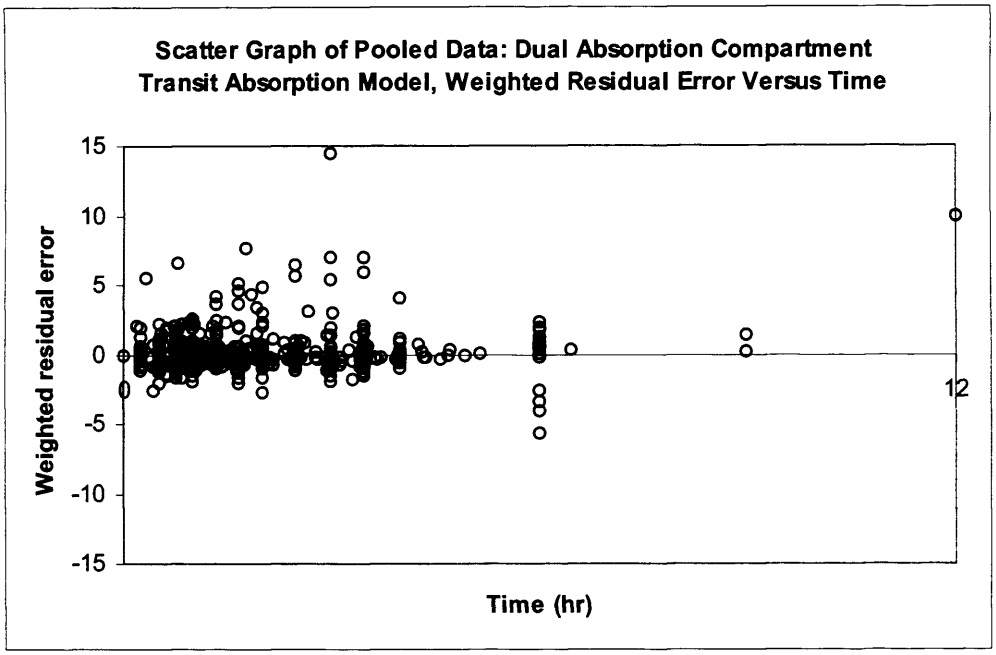
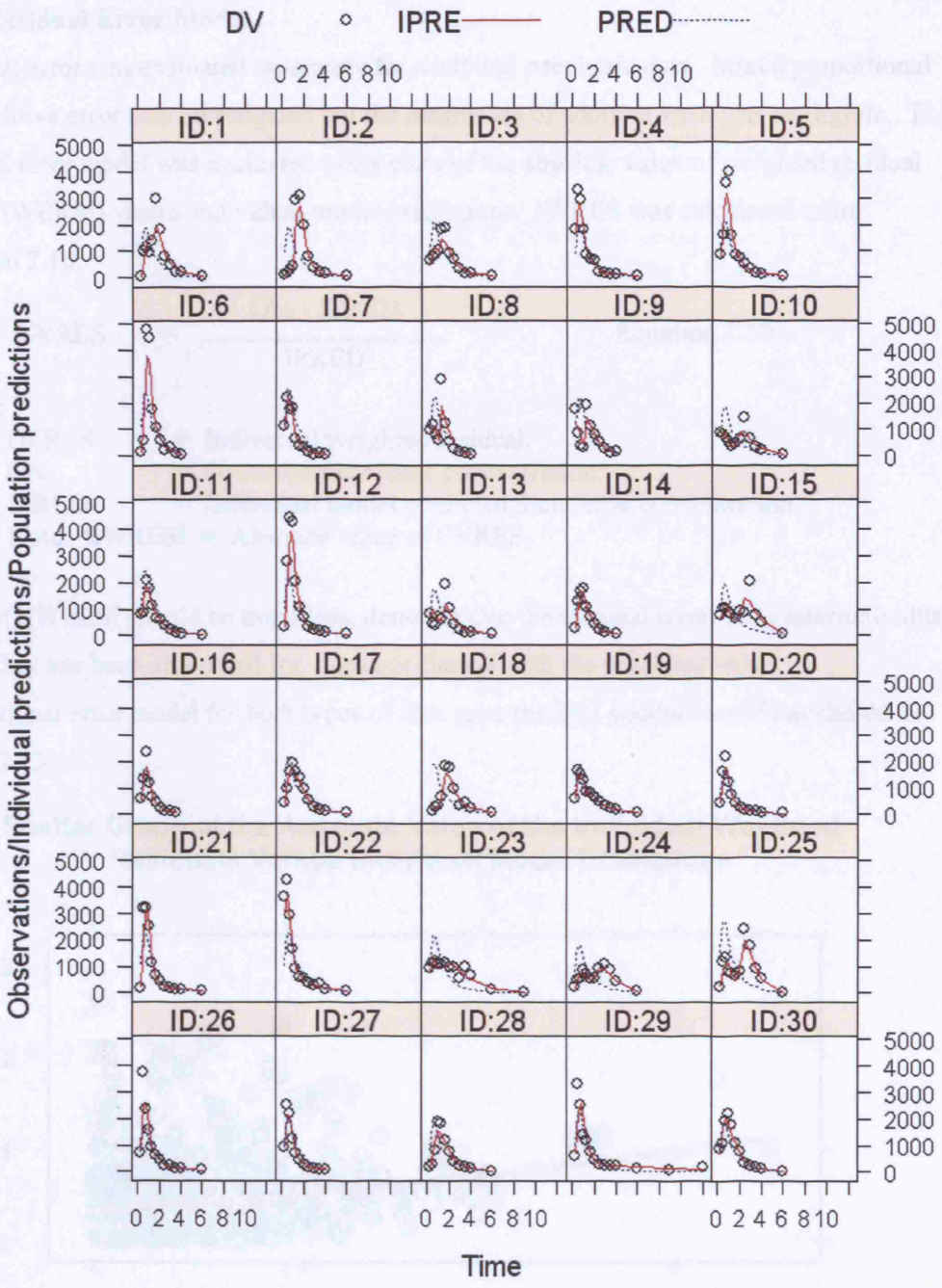


Figure 2.11: Scatter plot of weighted residual error versus time for final model.

Individual plots of model predictions and raw data are given in figure 2.12.



DV = Dependent variable (observed diclofenac concentration).
IPRE = Individual model prediction.
PRED = Population prediction.
Time = Time (hours).

Figure 2.12: Individual plots of adult data from pooled analysis.

2.5.5 Residual Error Model

Residual error was estimated separately for adult and paediatric data. Mixed proportional and additive error was investigated but the magnitude of additive error was negligible. The residual error model was evaluated using plots of the absolute value of weighted residual error (|IWERS|) versus individual model predictions. IWRES was calculated using equation 2.10:

$$IWRES = \frac{Obs - IPRED}{IPRED} \qquad \text{Equation 2.10.}$$

Where:

IWRES = Individual weighted residual.
Obs = Observed diclofenac concentration.
IPRED = Individual model predicted diclofenac concentration.
Note: |IWRES| = Absolute value of IWRES.

Plots of |IWRES| should be trend-less, denoting that the residual error, once interindividual variability has been accounted for, does not change with the predicted value. A proportional error model for both types of data gave the best goodness-of-fit as shown in figure 2.13.

Scatter Graph of the Absolute Value of the Individual Weighted Residuals Versus Individual Model Predictions

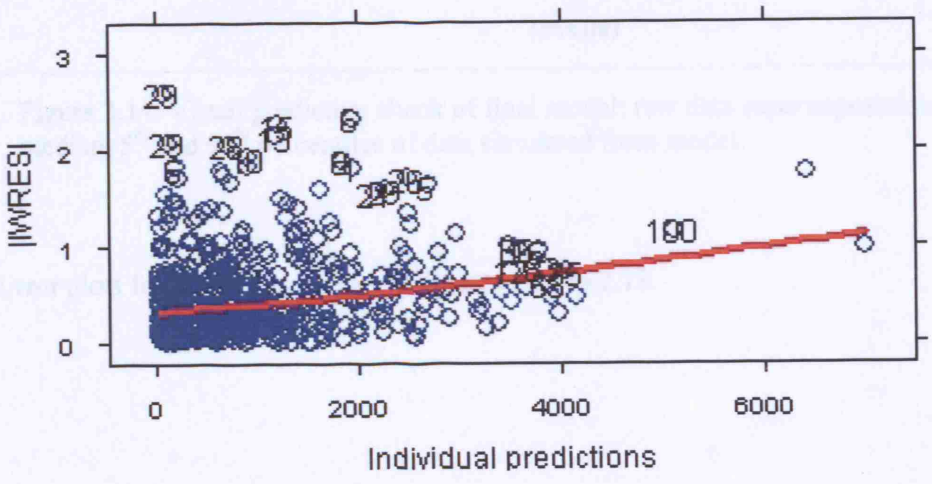


Figure 2.13: Absolute individual weighted residuals versus individual predicted diclofenac concentrations for final model.

2.5.6 Model Evaluation

The visual predictive check is given in figure 2.14.

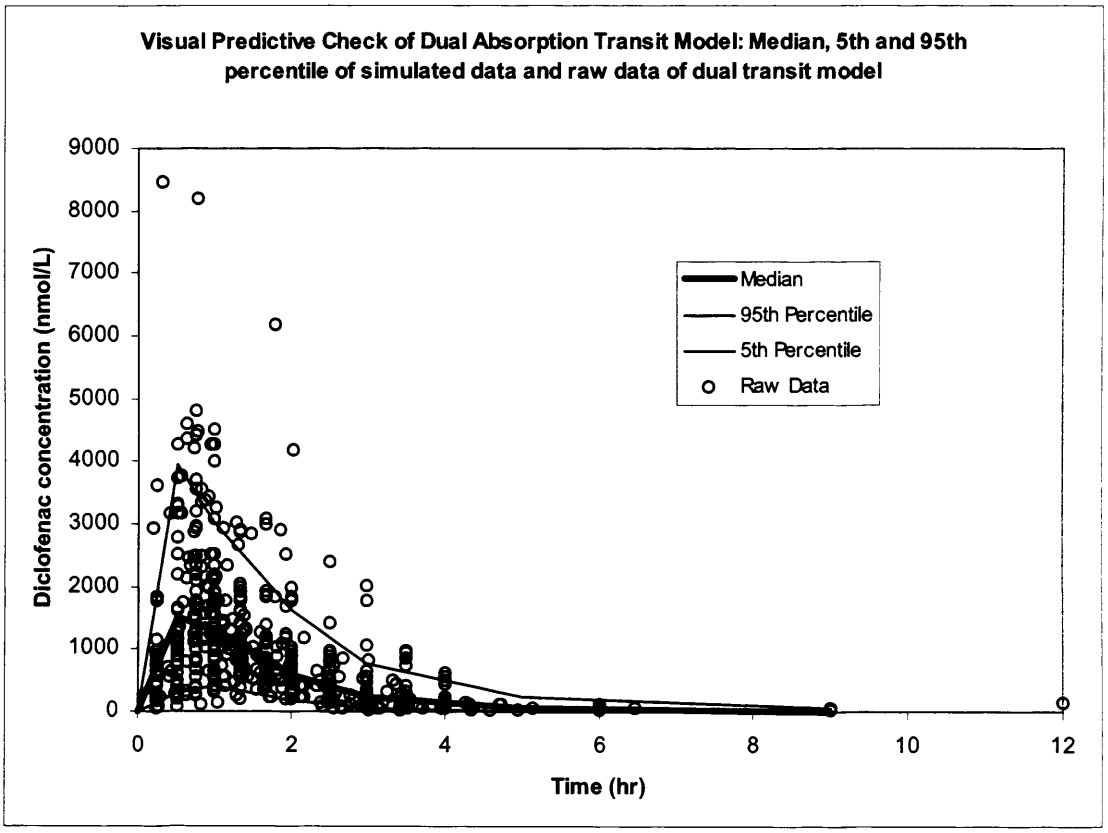


Figure 2.14: Visual predictive check of final model: raw data superimposed on median, 5th and 95th percentiles of data simulated from model.

Mirror plots from Xpose are given in figures 2.15 to 2.18.

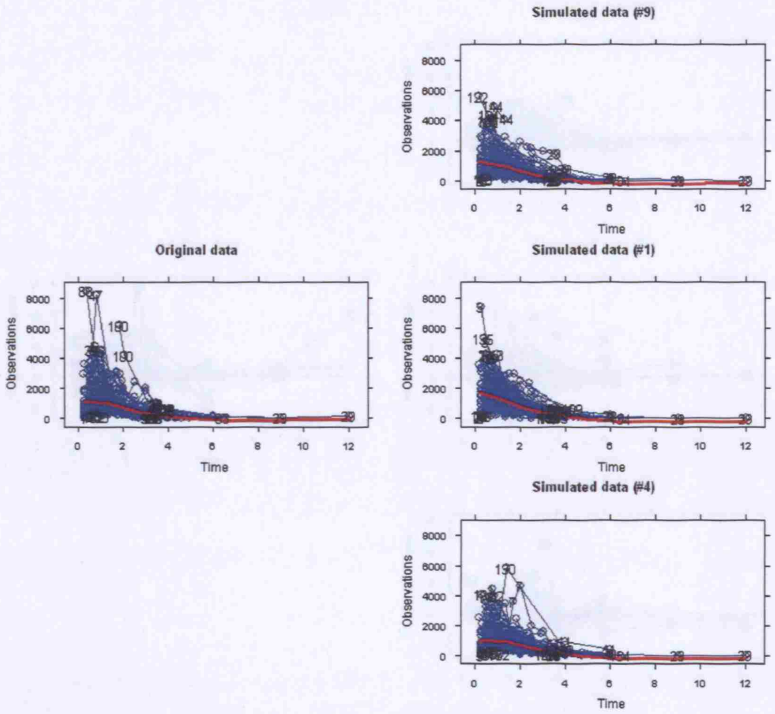


Figure 2.15: Mirror plots comparing original raw data with simulated data.

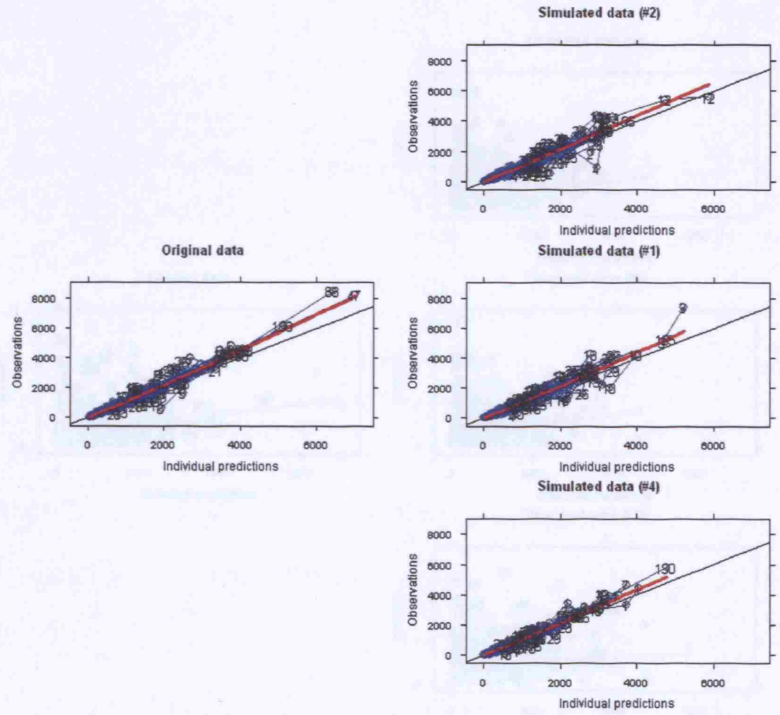


Figure 2.16: Mirror plots comparing original data individual predictions versus observations compared with simulations.

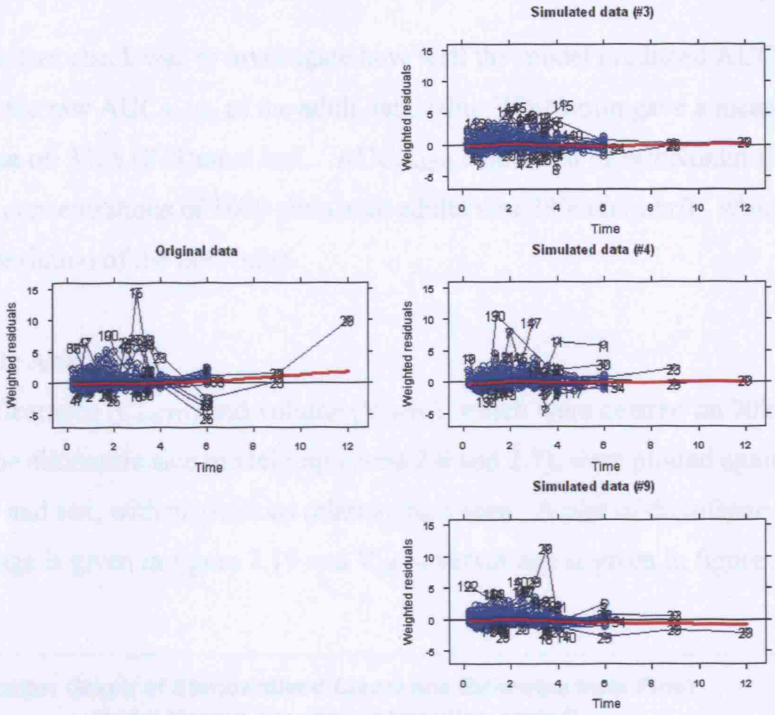


Figure 2.17: Mirror plots comparing original data weighted residuals versus time compared with simulations.

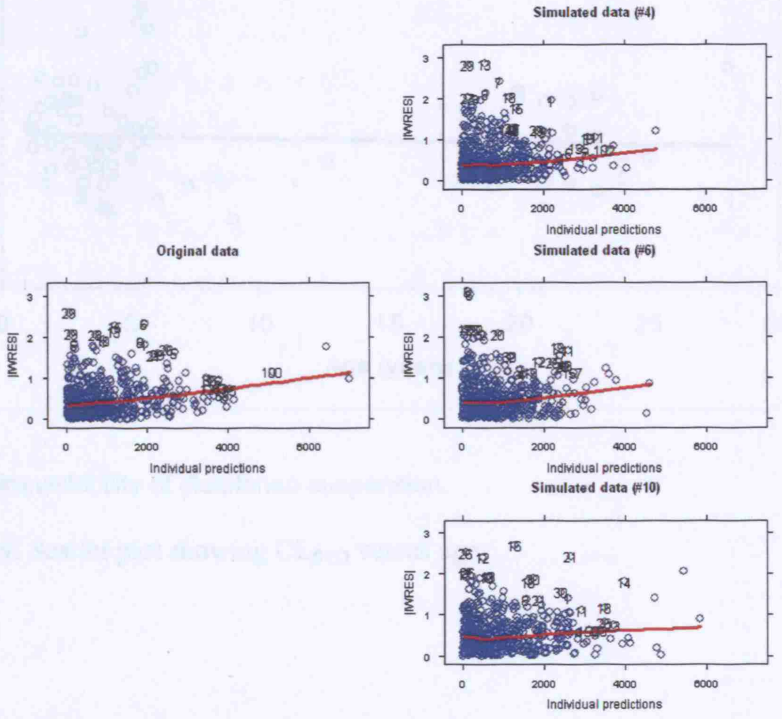
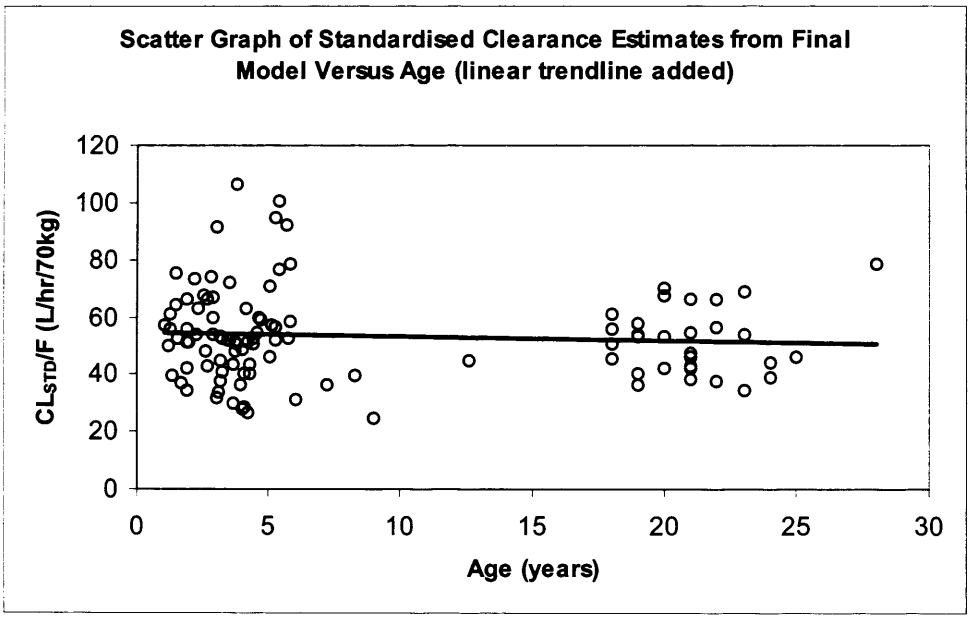


Figure 2.18: Mirror plots comparing original data absolute weighted residuals versus individual predictions compared with simulations.

The final predictive check was to investigate how well the model predicted $AUC_{(0\text{-}12h)}$. Calculation of the raw $AUC_{(0\text{-}12h)}$ of the adult data using WinNonlin gave a mean (standard deviation) value of: 3368 (879)nmol.hr/L. $AUC_{(0\text{-}12h)}$ calculated in WinNonlin from the median serum concentrations of 3000 simulated adults was 2806nmol.hr/L, which is within one standard deviation of the raw value.

2.5.6 Covariate Analysis

Standardised clearance (CL_{STD}) and volume (V_{DSTD}), which were centred on 70kg according to the allometric size model (equations 2.6 and 2.7), were plotted against age, weight, height and sex, with no obvious relationships seen. A plot of diclofenac suspension CL_{STD} versus age is given in figure 2.19 and V_{DSTD} versus age is given in figure 2.20.



F = Oral bioavailability of diclofenac suspension.

Figure 2.19: Scatter plot showing CL_{STD} versus age.

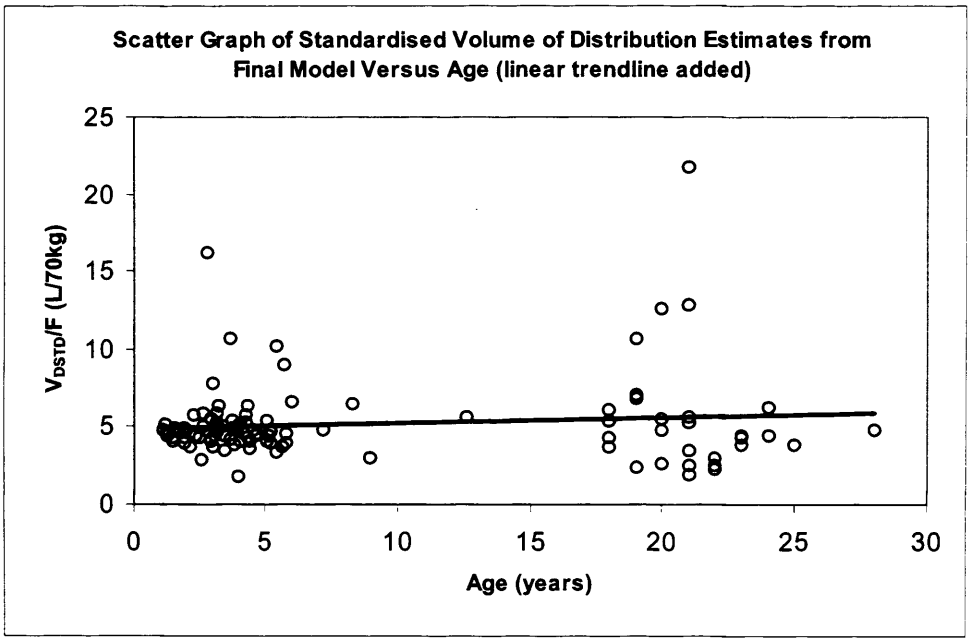
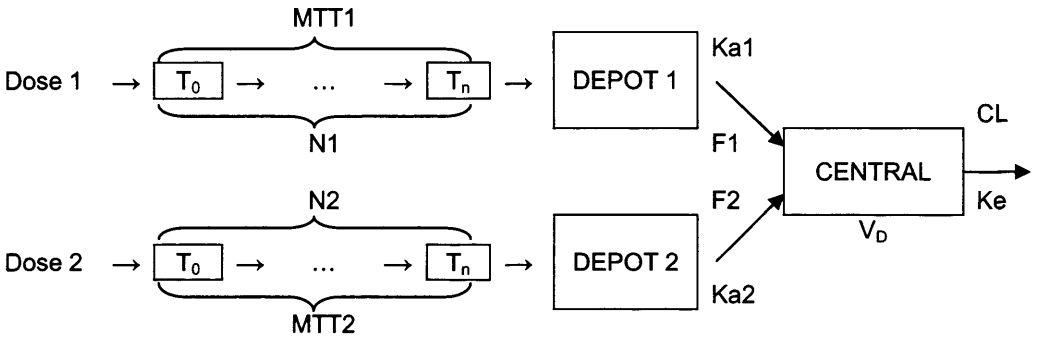


Figure 2.20: Scatter plot showing V_{DSTD} versus age.

Geometric mean CL_{STD}/F values of 52.9, 50.8 and 50.4 L/hr/70kg were calculated for children aged 1-3, 4-12 and adults respectively.

2.5.7 Final Parameter Estimates

A schematic diagram of the final model is given in figure 2.21, and final parameter estimates in table 2.2. The NONMEM control file of the final model is given in Appendix 7.5.



Tn = Transit compartment.

The following fixed effects were estimated in NONMEM:

MTT1 = Mean transit time into first depot compartment (hr).
N1 = Number of transit compartments prior to first depot compartment.
F1 = Fraction absorbed from first depot compartment.
$t_{1/2A1}$ = Absorption half-life from first depot compartment (hr) = ln2/Ka1.
MTT2 = Mean transit time into second depot compartment (hr).
N2 = Number of transit compartments prior to second depot compartment.
F2 = Fraction absorbed from second depot compartment (fixed to = 1 – F1).
$t_{1/2A2}$ = Absorption half-life from second depot compartment (hr) = ln2/Ka2.
V_D = Volume of distribution (L).
CL = Clearance (L/hr) = V_D x Ke.

Figure 2.21: Schematic diagram of final model and overview of fixed-effects estimated in NONMEM.

Table 2.2: NONMEM parameter estimates from final model.

Fixed effects (θ)		**Random effects (η)**	
Parameter	**Estimate**	**Inter-individual variability (%)**	**Between occasion variability (%)**
MTT1 (hr)	0.68	82	-
N1	1.03	102	-
F1	0.70	24	-
$t_{1/2A1}$ (hr)	0.09	31	-
MTT2 (hr)	1.37	117	-
N2	41.60	147	-
$t_{1/2A2}$ (hr)	1.06	49	-
V_D/F (L/70kg)	4.84	54	93
CL/F (L/hr/70kg)	53.98	26	20
Residual variability (ε) (%):			
Adult data:		29	
Paediatric data:		18	

2.5.8 Simulations

The results of simulated population $AUC_{(0\text{-}12h)}$ values at different dosing levels compared with the adult 50mg value are given in table 2.3. This shows that in children 1mg/kg gives the closest $AUC_{(0\text{-}12h)}$ value to 50mg in adults

Table 2.3: Simulated AUC values for dosing between 0.5mg/kg and 2mg/kg.

Patient age group	**Dose**	**$AUC_{(0\text{-}12h)}$ /F (nmol.hr/L)**	**Ratio (Paediatric $AUC_{(0\text{-}12h)}$/ Adult 50mg $AUC_{(0\text{-}12h)}$)**
Adult	50mg	2793.06	-
Child 1-3 years	0.5mg/kg	1391.58	0.50
Child 1-3 years	1mg/kg	2788.05	1.00
Child 1-3 years	1.5mg/kg	4174.76	1.50
Child 1-3 years	2mg/kg	5566.32	1.99
Child 4-6 years	0.5mg/kg	1545.82	0.55
Child 4-6 years	1mg/kg	3022.02	1.08
Child 4-6 years	1.5mg/kg	4637.45	1.66
Child 4-6 years	2mg/kg	6183.30	2.21
Child 7-12 years	0.5mg/kg	1633.65	0.59
Child 7-12 years	1mg/kg	3301.75	1.18
Child 7-12 years	1.5mg/kg	4900.95	1.76
Child 7-12 years	2mg/kg	6534.59	2.34

2.6 Discussion

This study has shown that diclofenac 1mg/kg in children produced similar exposure to 50mg in adults, and that variability in CL and V_D is adequately explained by the allometric size model. The recruitment rate was better than expected and shows that it is possible to undertake clinical drug trials involving extra blood sampling in children with few problems. The major reason for such a high recruitment rate was probably that the study was designed around clinical practice, blood sampling was usually undertaken in the operating theatre and parents were free to decline the final blood sample if the child was unduly distressed in the post-operative period. Another factor that aided recruitment was that diclofenac suppositories would routinely be used regardless of whether the child entered the study, so there was unlikely to be any difference in safety by taking part. Patients did receive a digital watch for participating in the study, but this was not advertised to them before recruitment and formed a (pleasant) surprise on discharge from hospital.

Raw plots of diclofenac concentration versus time showed double peaks in some adult patients, and although only a maximum of three samples per dose were observed in the paediatric patients, it was clear that for some patients a similarly complex absorption process was occurring (figures 2.7 & 2.8). For this reason, the dual absorption compartment model was chosen, as it provided the flexibility to predict both single and double peaks, as evidenced in the individual plots (figure 2.12) where it can be seen that individual predictions track the double peaks seen in the adult data. As discussed in Chapter One, the most likely explanation for these double peaks is pH dependent dissolution, with variability in the pH of sections of the small intestine meaning that diclofenac enters and leaves solution, its availability for absorption varying similarly. Double and multiple peaks are a phenomenon frequently seen with immediate release diclofenac (Chan KKH et al, 1990, Lotsch J et al, 2000, Macia MA et al, 1995), and their presence in this study was not unexpected.

The final model used for this study was the first to apply transit compartment absorption to diclofenac and possibly the first to apply transit absorption to a dual absorption compartment model. The majority of published papers on diclofenac pharmacokinetics used traditional non-compartmental modelling, as would be expected for a drug licensed in 1974. However, there were two population pharmacokinetic studies which did apply compartmental models and from which ideas for the present study were taken. One previous attempt (Lotsch J et al, 2000) at compartmental modelling of immediate release diclofenac managed to fit a two-compartment model to soluble tablet data but was unable to estimate interindividual variability for any of the fixed effects. The same study also included data on conventional (not enteric-coated) tablets but failed to obtain a population model to fit the data. Raw data presented in this study showed the presence of double/multiple peaks for both formulations, highlighting the need for better structural models to be tested, especially where more complex absorption profiles are seen. This study also highlights the need for thorough review of the literature before embarking on any research, as two years previously a paper was published showing that the dual absorption compartment model provides a good fit for diclofenac data containing double peaks (Idkaidek NM et al, 1998). A dual absorption with peripheral disposition compartment model (as in figure 2.1 model 4) was used, which consisted of only rich adult data on

sustained release, enteric-coated and suppository formulations (Idkaidek NM et al, 1998). No peripheral compartment was included in the final model for the present study as its addition did not seem to improve goodness-of-fit plots, and successful minimisation was not obtained. As little absorption and distribution phase data was available in the paediatric patients, it is perhaps unsurprising that a single disposition compartment model adequately described the data.

The raw plots (figures 2.7 and 2.8) show that two paediatric patients had much higher (almost two-fold) diclofenac concentrations than any of the adults, and that one paediatric patient had a delayed/second peak that was higher than seen in the adults. One explanation for this could be that the paediatric patients received a slightly higher dose than the adults: 1mg/kg versus approximately 0.7mg/kg (=50mg/mean adult weight). This 30 percent difference in dose however would not account for such large differences in concentration; from dose escalation studies in adults C_{max} would be expected to increase by 30 percent with a 30 percent dose elevation (Lau HSH et al, 1989). It may be the case that paediatric patients have a higher gastrointestinal pH than adults, causing more rapid dissolution, increasing absorption rate and causing higher peak concentrations. However, gastric pH levels should be adult equivalent by two years of age (Morselli PL et al, 1980, Kearns GL et al, 2003, Sreedharan R & Mehta DI, 2004) and only one of the outlying patients was under this age, the other two being aged four and nine years. The possibility that assay variability contributed to these high paediatric values, adult and paediatric samples having been processed in different laboratories, cannot be discounted although it is unlikely given that a similar analytical method was used. Another possible explanation for the seemingly more erratic absorption in the paediatric patients is that they underwent a surgical procedure, whereas the adult volunteers did not. This would certainly explain the delayed peak concentration, which may be due to delayed gastric emptying caused by anaesthesia (Kennedy JM & van Riji AM 1998), but as the dose was generally given before surgery, no major systematic influence on the pharmacokinetic profile of diclofenac is envisaged.

The aim of the model building strategy was to explain the rich adult data well, and therefore to allow a more informative model to be fitted to the sparse (few samples per patient) paediatric data points. In simple terms, NONMEM was given a relatively complex

model supported by the rich adult data, and asked to derive parameter estimates that would explain the observations in children. For some of the paediatric patients, no absorption phase data was available as all samples were taken after C_{max} had been reached. In this instance, NONMEM ascribed a value close to the population estimate for absorption parameters in these individuals, but would be able to estimate CL reasonably well. However, the presence of sparse data leads to the risk of shrinkage of the random effects. Shrinkage is a term used to describe the phenomenon of the distribution of random effects shrinking towards the observed value, and occurs where there are few samples per individual; such individuals provide relatively little information to the population estimate meaning that the individual predictions (model predictions based on both the population parameter and the estimate of variability) tend to be similar to the observed value (Karlsson MO & Savic RM, 2007). Figure 2.10 shows the individual predictions versus observations and it can clearly be seen that the paediatric data falls closer to the line of unity than adult data, indicating shrinkage has occurred. This means that the plots of individual prediction versus observation look falsely good, and should not be relied upon to determine the usefulness of the model.

Model evaluation prior to dose predictions therefore mainly relied on simulation-based diagnostics. These techniques compare data simulated from the final model with the original data; how similar they are is a good measure of model performance, and as they do not rely on the empirical Bayes estimates from the NONMEM posthoc step, are not prone to bias induced by shrinkage. The simulation-based evaluations showed that the model was reasonably good at predicting the original data. Figure 2.14 shows the visual predictive check of the final model, with most raw data points falling within the boundaries of the simulated data. Mirror plots from Xpose showed the model was able to simulate similar data with a similar distribution to the raw data (figures 2.15-2.18), and that standard diagnostic plots for the simulated data were also similar to those derived from the original data. The most important evaluation was the finding that model simulated $AUC_{(0\text{-}12h)}$ values were within one standard deviation of the actual mean value calculated by non-compartmental analysis. As this study used $AUC_{(0\text{-}12h)}$ as a surrogate marker of efficacy, this check gave confidence that the model performed well enough to predict $AUC_{(0\text{-}12h)}$ values at different doses. The final population parameter estimate (table 2.2) for CL was

similar to a previous study on diclofenac suppositories in children (van der Marel CD et al, 2004); this study estimated a CL_{STD}/F of 44.82 L/hr/70kg compared with 53.98 L/hr/70kg estimated for the present study, with the possibility of slightly lower suspension bioavailability accounting for the difference.

A possible criticism of this study could be that adult data was discarded if it fell below the LOQ, and this constituted many of the samples taken after four hours post-dose. It has been shown that omitting data below the LOQ can cause small biases in fixed effect estimates (Hing JP et al, 2001), but the proposed solutions such as substituting values with half the LOQ are also imperfect. The ideal method to deal with below LOQ data is to include all concentrations, regardless of where they lie in relation to the LOQ and model assay variability in NONMEM as a random effect. For the present study this was not possible, as raw results from below the LOQ were not provided, and so these points were omitted with the awareness that some bias may appear (Duval V & Karlsson MO, 2002).

To investigate the ontogeny of diclofenac pharmacokinetics, covariate analysis focussed on how and whether CL and V_D varied with age. Figures 2.19 and 2.20 show that model estimations for standardised CL and V_D do not vary with age and suggest that allometric weight scaling adequately explains the changes in CL and V_D during development. As discussed in Chapter One, the allometric size model of CL varying with $w^{3/4}$ is based on the observation that basal metabolic rate scales with $w^{3/4}$ (Kleiber M, 1947). This study found that diclofenac CL could adequately be described by scaling estimates to $w^{3/4}$ over a ten-fold weight range (figure 2.19).

Once the final model had been evaluated and shown to predict both the original data and $AUC_{(0\text{-}12h)}$, it was possible to simulate new data where patients were given different doses, to investigate the most suitable paediatric dose. No pharmacodynamic markers of pain were collected in the paediatric patients for two main reasons. The first reason was that it was envisaged that recruitment may be difficult and the primary aim was to describe diclofenac pharmacokinetics. If a pharmacodynamic measure of pain, such as pain scoring was included in the study, it would have been necessary to standardise the operative procedure, anaesthetic technique and administration of supplemental analgesics to reduce

confounding factors. This would have severely limited the number of patients eligible for recruitment and made the study more onerous to carry out. The second reason that pain scoring was not undertaken is that its reliability in children of different ages is questionable. The specificity of respiratory and cardiovascular responses to pain is poor meaning they cannot be relied on to provide analgesic efficacy information. In addition, infants and young children usually require observational-type pain scoring rather than self-reporting, which can be used at age five years and over (Lloyd-Thomas AR, 1999). For these reasons it was decided to use a surrogate for an effective dose: the $AUC_{(0\text{-}12h)}$ produced when adults are given 50mg of diclofenac. As discussed in Chapter One (figures 1.3 & 1.4) diclofenac 50mg is almost twice as effective as 25 mg but similarly effective to 100mg (McQuay HJ & Moore RA, 1998). Diclofenac's linear pharmacokinetics in this dosing range (Lau HSH et al, 1989) means $AUC_{(0\text{-}12h)}$ will double when dose doubles, so the pharmacodynamic data suggests a ceiling effect above 50mg. This ceiling effect probably indicates receptor (mainly COX-2) saturation, which diclofenac inhibits in a time-dependent manner *in vitro* (Blobaum AL & Marnett LJ, 2007, Rowlinson SW et al, 2003). This mixture of *in vivo* adult pharmacodynamic data and diclofenac *in vitro* data formed the theoretical basis that an $AUC_{(0\text{-}12h)}$ similar to 50mg in adults would be effective in children, and in the absence of reliable paediatric pain scoring systems, seemed a reasonable assumption.

A useful feature in NONMEM is the ability to simulate new data based on parameter estimates from a pharmacokinetic model. In addition to their use in model evaluation, pharmacokinetic predictions of altered dosing can also be undertaken. Table 2.3 shows that 1mg/kg gave a similar $AUC_{(0\text{-}12h)}$ to 50mg in adults. Linear (mg/kg) dose schedules will lead to increased $AUC_{(0\text{-}12h)}$ in older (heavier) children as diclofenac CL was adequately described using the allometric $w^{3/4}$ model, although for diclofenac this approximate 20 percent difference between infants and children aged seven to 12 years is unlikely to be of clinical significance. The mg/kg dosing schedule was recommended instead of age banding (which would have been perfectly acceptable from an efficacy and safety point of view) because this is the system most commonly used in paediatric hospitals. Age banding tends to result in different drugs being banded by different age stratifications depending on the results of pharmacokinetic studies. The mg/kg system may not be ideal in that CL does not follow a linear pattern, but it does provide a simple, easy-to-remember formula by which

paediatric health professionals can calculate the dose. Furthermore, the suspension formulation gives the flexibility to administer various doses to different aged children.

2.7 Conclusions

The main finding of this study was that the paediatric dose that should give the most similar effect to 50mg in adults is 1mg/kg. The recommended dose in children over six years for acute post-operative pain is 1-2mg/kg/day in divided doses limited to four days treatment (*Diclofenac SPC*, 2005). In adults diclofenac is given eight-hourly and no such limit on duration of treatment is set. The results of this study suggest that the current dosing guidelines for children aged 7-12 are too low: three doses of 50mg in adults would give a cumulative AUC of 8379nmol.hr/L whereas 2mg/kg/day in children aged 7-12 would give a daily AUC of 6604nmol.hr/L giving a paediatric:adult AUC ratio of 0.79, and if the same dose recommendation was made for younger children, the one to three year-olds would only receive a fraction of 0.67 of the adult daily dose. Assuming that target exposure is important for efficacy, and as doubling the AUC (Lau HSH et al, 1989) almost doubles efficacy in the range 25 to 50mg in adults (McQuay HJ & Moore RA, 1998), it would seem that the current recommended paediatric dose is slightly low, and although 1mg/kg three times daily may lead to a slightly higher (approximately 20 percent) exposure in children aged seven to 12 years, it is unlikely to be of clinical significance.

The second interesting conclusion is that the allometric model of $w^{3/4}$ on CL and w on V_D adequately explained the parameter variation from one year-olds through to adults (figures 2.19 & 2.20). This suggests that any differences in diclofenac pharmacokinetics between children aged one year and over and adults can adequately be explained by differences in body size. Compared with the effect of body size, any theoretical variability caused by maturational factors such as development of renal function, metabolic capacity, differences in body fat content as mentioned in many paediatric pharmacology references and summarised in Chapter One of this thesis, are small enough to be insignificant in terms of diclofenac dosing.

Chapter THREE: Types of Common Adverse Drug Reactions to Diclofenac for Peri-Operative Pain in Children

3.1 Introduction

The term adverse drug reaction will be used throughout this thesis. Whilst strictly an adverse drug reaction pertains to an unwanted effect of a drug, its use here will more broadly refer to adverse reactions to medicines, including their excipients. Several definitions of adverse drug reaction have been proposed, a recent example being:

> "An appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product." (Edwards IR & Aronson JK, 2000).

Such definitions contain several terms open to ambiguity. A sensation of nausea may or may not be considered "appreciably harmful or unpleasant", or warrant discontinuation of the medicine, depending on the context. For example, a life-saving cytotoxic agent would probably not be withdrawn if it caused nausea, and nausea may not be considered appreciably harmful compared with other potential effects. Perhaps in this context nausea would not be classed as an adverse drug reaction using this definition, whereas nausea caused by an analgesic used for a headache may be considered appreciably harmful causing the patient to stop taking the medication. For the purpose of this thesis, an adverse drug reaction will be defined as:

> "An adverse event caused by a medicinal product."

This contains three possible sources of ambiguity, namely "adverse event", "caused" and "medicinal product".

An adverse event is any untoward occurrence that presents during drug treatment, regardless of its cause. For this study, the term adverse event will be an adapted form of a common definition (Finney DJ, 1965):

> "Any new diagnosis, reason for referral to a doctor, unexpected deterioration in a concurrent illness, any suspected adverse drug reaction, or any other complaint considered to be of sufficient importance to enter in the patient's medical/nursing notes. Any laboratory test result outside the usual reference range and any complaint from the patient or parent will also be classified an adverse event."

Whilst it may seem that this definition is likely to include a broad range of events, some of which bear no relationship to the administration of a medicinal product, the importance of collecting such data was highlighted during events that led to the withdrawal of practolol in the 1970s. This β-adrenergic blocking agent was found to cause an oculomucocutaneous syndrome probably triggered by an immunologic reaction to a metabolite (Amos HE, 1979). This reaction caused sclerosis of membranes, especially in the eye, and was also responsible for at least 40 deaths due to sclerosing peritonitis. The relationship between practolol and ocular complications was made by ophthalmologists working at Moorfields Eye hospital in London who developed a system of centralised monitoring for ocular disease; this was after several years of serious complications associated with practolol going undetected as they were simply unexpected reactions to a β-blocker (Abraham J & Davis C, 2006). Recording and reporting of all adverse events rather than just suspected adverse drug reactions in clinical studies allows for the epidemiological detection of such unexpected effects in a more timely fashion (Skegg DCG & Doll R, 1977).

The second term requiring clarification in the definition of an adverse drug reaction is the word "caused". This study will be observational so the cause of adverse events cannot be simply inferred from comparisons with a control group. The observational study design is often the best way to gain quantitative information on adverse drug reactions (Kaufman DW & Shapiro S, 2000), but it is necessary to use a systematic process in determining how likely it is that adverse events are adverse drug reactions. In order to do this, causality assessment will be used. The World Health Organisation (WHO) has produced a series of definitions of terms used in causality assessment, the details of which are given in figure 3.1.

WHO categories of adverse event causality:

Very likely/Certain:
A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probable/likely:
A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfil this definition.

Possible:
A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug, but which could also be explained by current disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Unlikely:
A clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanation.

Unrelated:
A clinical event, including laboratory test abnormality, with an incompatible time relationship to drug administration, and which could be explained by underlying disease or other drugs or chemical.

Unclassifiable:
A clinical event, including laboratory test abnormality, with insufficient information to permit assessment and identification of the cause

Figure 3.1: WHO Causality Categories.
From: Edwards IR & Aronson JK, 2000

The first causality assessment tool used to aid in the process of attributing adverse events to adverse drug reactions (Hutchinson TA et al 1979, Kramer MS et al, 1979, Leventhal JM et al, 1979) was developed in response to the growing realisation that experts in clinical pharmacology could not agree on whether the same adverse event in the same patient was an adverse drug reaction. This laborious multiple question algorithm was found to give similar results (Michel DJ & Knodel LC, 1986) to a much shorter ten-point scoring system (Naranjo CA et al, 1981) (figure 3.2). This scoring system by Naranjo and an algorithm based on yes/no questions centring on temporal relationship and rechallenge (Jones JK, 1982) (figure 3.3) were used in a large prospective study on adverse drug reactions as a cause of hospital admissions (Pirmohamed M et al, 2005). The authors of this study

recognised that the reasonableness of temporal relationships and presence or absence of other potential causative factors, which tend to be the key factors upon which causality algorithms are based, are still open to interpretation, and so each adverse event was finally classified according to the opinion of an expert in adverse drug reaction detection. An important point to note at this stage is that determining causality of adverse events cannot easily be formulated into a simple algorithm, but that such algorithms can certainly be used to separate out the events that are unlikely to be related to a drug due to temporal association, and can also be used to guide a systematic thought process in attributing causality.

Naranjo causality assessment

To assess the adverse drug reaction, please answer the following questionnaire and give the pertinent score

	Yes	No	Do not know	Score
1. Are there previous *conclusive* reports on this reaction?	+1	0	0	
2. Did the adverse event appear after the suspected drug was administered?	+2	-1	0	
3. Did the adverse reaction improve when the drug was discontinued or a *specific* antagonist was administered?	+1	0	0	
4. Did the adverse reaction reappear when the drug was readministered?	+2	-1	0	
5. Are there alternative causes (other than the drug) that could on their own have caused the reaction?	-1	+2	0	
6. Did the reaction occur when a placebo was given?	-1	+1	0	
7. Was the drug detected in the blood (or other fluids) in concentrations known to be toxic?	+1	0	0	
8. Was the reaction more severe when the dose was increased, or less severe when the dose was decreased?	+1	0	0	
9. Did the patient have a similar reaction to the same or similar drugs in *any* previous exposure?	+1	0	0	
10. Was the adverse event confirmed by any objective evidence?	+1	0	0	
			Total score	_____

Definite: ≥9
Probable: 5 to 8
Possible: 1 to 4
Doubtful: ≤0

Figure 3.2: Naranjo Causality Assessment.
From: Naranjo CA et al, 1981

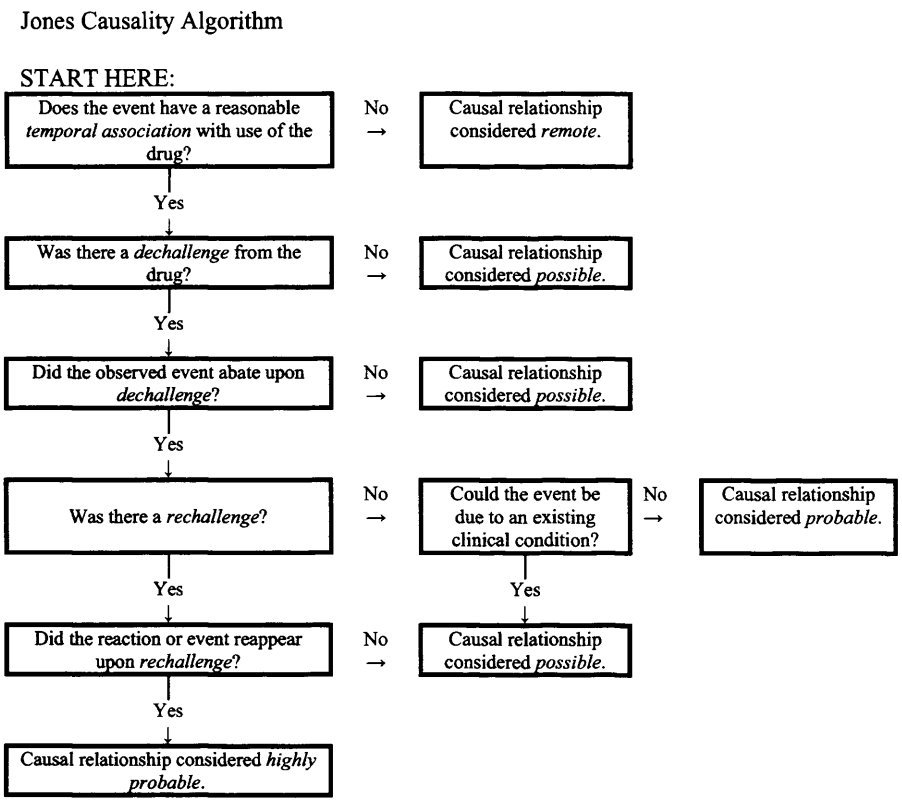


Figure 3.3: Jones Causality Assessment.
From: Jones JK, 1982.

The final term to be defined is "medicinal product". A medicinal product is defined in the UK Medicines Act 1968 as being:

> "Any substance or article (not being an instrument, apparatus or appliance) which is being manufactured, sold, supplied, imported or exported for use wholly or mainly in either or both of the following ways, that is to say:
>
> a) use by being administered to one or more human beings or animals for a medicinal purpose;
>
> b) use as an ingredient, by a practitioner or in a pharmacy or in a hospital or in a business comprising the sale of herbal remedies, in the preparation of a substance or article which is to be administered to one or more human beings or animals for a medicinal purpose." (*MEP*, 2006)

From this definition it can be seen medicinal product encompasses not just diclofenac (the drug) but the entire formulation including excipients. Any diclofenac-containing product used for systemic treatment of acute pain in the peri-operative period (any point in the hospital admission for a surgical procedure) will be included, and will usually be intravenous or intramuscular injection, suppository, enteric-coated tablet, soluble tablet or oral suspension.

This study will investigate the common adverse drug reactions of diclofenac, the term commonly being defined as occurring in greater or equal to one percent of patients. Usually common adverse reactions are relatively minor and do not cause significant morbidity. However, for patients and parents these effects can be disturbing and may affect compliance with future treatment. It is therefore important to ascertain the types of common adverse reactions, so that patients and parents can be advised in advance of potential effects, and so that informed choice of analgesia can be made.

In order to determine the potential for morbidity and mortality of adverse events, each one will be given a measure of seriousness. The type, frequency and cause of serious adverse events are of great interest in clinical practice, as minimising iatrogenic harm is crucial. The definition of a serious adverse event to be used in this study is given in figure 3.4.

A serious adverse event is:
1. Fatal.
2. Life-threatening.
3. Prolongs hospitalisation.
4. Causes persistent or significant disability or incapacity.
5. Requires medical or surgical intervention to prevent the above (1-4)

Figure 3.4: Definition of a serious adverse event.
Adapted from: Edwards IR & Aronson JK, 2000.

3.2 Aims

To identify the type and frequency of diclofenac adverse drug reactions occurring in greater than one percent of paediatric patients.

To rigorously assess all serious adverse events and ascertain the likelihood of them being adverse drug reactions caused by diclofenac.

To compare the frequency of common adverse drug reactions to diclofenac in adults and children.

3.3 Objectives

To record all adverse events occurring in paediatric patients who receive diclofenac for acute pain relief in the peri-operative period.

To undertake a causality assessment to ascertain which adverse events are likely to be adverse drug reactions caused by diclofenac.

To rigorously assess all serious adverse events by having their causality reviewed by an expert panel.

3.4 Method

3.4.1 Type of Study and Sample Size

An intensive monitoring observational study of hospitalised children who were prescribed diclofenac for acute pain was undertaken. In this type of study, when n is the number of patients, events that were not seen must have an overall population frequency of less than 3/n patients with a confidence of 95 percent (Hanley JA & Lippman-Hand A, 1983). Therefore, in order to be 95 percent confident of detecting all reactions occurring in greater than one percent of patients, a minimum sample of 300 patients was required. This also ensured a 95 percent confidence that events not seen must occur in less than one percent of patients. It must be noted that with this relatively small number of patients, the overall population frequency of adverse drug reactions detected would only be estimated with large confidence intervals.

3.4.2 Recruitment

Written informed consent from parents, and where appropriate assent from patients, was required for this study as access to the medical notes and patient/parent questioning were

required. The decision to prescribe diclofenac was solely made by the doctors (usually the anaesthetist) under whose care the patient was. However, in order to minimise subject bias, patients were identified and recruited for the study prospectively, before surgery. Parents of children aged 12 years or under scheduled for surgery were approached in the pre-admission clinic, on admission to hospital prior to surgery, or by post with an information leaflet and stamped addressed envelope for the return of completed consent forms. The recruitment pack containing information leaflets, consent form and pre-paid envelope were sent out by the hospital secretaries with the admission letter. The inclusion criteria for entering the study were:

1. Patients aged 12 years or under at the time of the operation.
2. Written informed consent from parents to record data relating to adverse events; if appropriate, assent from patients was also obtained.
3. Patients who received diclofenac (at the discretion of the physician under whose care the child was) during the hospital admission were included in the analysis of adverse events.

The exclusion criteria were:

1. Patients aged 13 years or over at the time of diclofenac administration.
2. Patients admitted for emergency surgery or high-risk life-threatening surgery as giving the patient/parents advanced notice would not be possible in emergency situations. It was thought ethically unsound to ask the family to take part in this research study if patients underwent high-risk surgery due to the need for parent questioning in the post-operative period.
3. Any patients already enrolled in more than one other study: To ensure patients and parents are not overwhelmed by researchers during their hospital stay.

High-risk surgery was classified as any operation for which the patient would be admitted to intensive care in the post-operative period. In practice this meant excluding all patients undergoing major cardiac surgery in addition to any others for whom a post-operative intensive care bed was booked in advance.

The setting for this study was the paediatric surgical wards at Great Ormond Street Hospital for Children and University College London Hospital. Independent ethics committees at each hospital approved the study (Appendix 7.6), and parents and patients were given an approved information sheet explaining the study (Appendix 7.7 & 7.8). In order to avoid biasing the decision to prescribe diclofenac, an entry in the medical notes including a copy of the consent form was made only after the patient had received the first dose. This would then alert the ward staff that the patient was being monitored for adverse events. Where patients had been enrolled but did not received diclofenac, demographic data only were collected to investigate drug utilisation. It was decided at the start of the study that this group of patients would not provide an adequate control group, so no information on adverse events was collected from them. The reason that these patients would not be an adequate control group is that the decision not to prescribe diclofenac would cause systematic bias. For example, such patients may be scheduled for more major procedures with high risk of adverse events, meaning the adverse event rate could be higher than in the diclofenac group undergoing more minor procedures; patients not receiving diclofenac may require more or higher doses of other analgesics, changing the adverse event rate; or diclofenac may be thought unnecessary for very minor procedures and so not prescribed, making these patients likely to have fewer adverse events. It was clear that these patients were unpredictably and systematically different from those who were prescribed diclofenac, and so inferences made from differences in adverse event rates could not be relied upon. For this reason causality assessment of adverse events was used.

3.4.3 Adverse Event Monitoring

All data was recorded on a case report form (Appendix 7.9). Allocating each patient with a unique code number ensured the case report forms were anonymised. The medical notes, with clarification if required from the parents, were used to compile detailed demographic information including past medical history, history of any previous allergies, the procedure undertaken, and by which surgeon and anaesthetist. The medical notes supplemented by parent questioning where necessary were also used to obtain a recent drug history and the inpatient drug charts and anaesthetic charts were used to collect details of all drugs and doses received during the admission. The medical notes, nursing notes and electronic reporting systems (for pathology results) were used to collect information on any adverse

events, with clarification from clinical staff where necessary. Patients and parents were also approached on the day after the operation (before discharge for day-case patients) on day three after the operation, and weekly thereafter and asked a single open question: Have you/your child experienced any problems? Time constraints meant that patients could not be visited daily and so this system was a reasonable compromise to ensure any events not recorded in the medical or nursing notes were found. Medicines prescribed on discharge were recorded and patients/parents were telephoned approximately one week after discharge to check for adverse events since leaving hospital. Medicines taken in the corresponding period after discharge were also noted.

3.4.4 Causality Assessment

Each adverse event was given a measure of causality using the WHO definitions (figure 3.1). In order to guide the assessment, the Naranjo (Naranjo CA et al, 1981) and Jones (Jones JK, 1982) algorithms (figures 3.2 and 3.3) were used, as in a recent adverse drug reaction monitoring study (Pirmohamed M et al, 2005). In addition, all serious adverse events were assessed by an expert panel consisting of: a paediatric anaesthetist, a paediatric clinical pharmacologist, and a paediatric pharmacist.

3.4.5 Drug Utilisation

Detailed demographic information including past medical history and reason for admission was recorded for patients who did not receive diclofenac. Comparisons of this demographic data with the group who did receive diclofenac were made to investigate drug utilisation.

3.4.6 Pilot Study

A pilot study on the urology ward of Great Ormond Street Hospital was undertaken to test the feasibility of recruiting patients prospectively and to refine the case report form contents. Full demographic details and adverse event monitoring was undertaken for patients recruited to the pilot study who received diclofenac, and so they were included in the overall analysis of adverse events.

3.4.7 Additional Adverse Event Data from the Pharmacokinetic Study

Exactly the same demographic, medical and adverse event data was collected from the patients participating in the pharmacokinetic study (Chapter Two). The information on these patients was combined with the patients in this study for the analysis of adverse events.

3.4.8 Data Analysis

Data from the case report forms was entered in to a demographic and adverse events database using the statistical package SPSS (version 13).

3.5 Results

3.5.1 Recruitment

The need for prospective written informed consent meant that not all patients scheduled for surgery were recruited to the study. Patients who were not scheduled for pre-admission clinic were sent a recruitment pack with a pre-paid envelope with the hospital admission letter. Of 650 recruitment packs sent out, 137 signed consent forms were returned. Most patients (252 of 407 approached) were recruited in person from either the pre-admission clinic or on admission to hospital. A consent rate cannot be accurately calculated as many of the patients recruited in person had also been sent a recruitment pack. Reasons for not consenting were not systematically recorded as it was a statutory requirement of the ethics committee that reasons for not consenting did not have to be given. A schematic diagram of patient recruitment is given in figure 3.5.

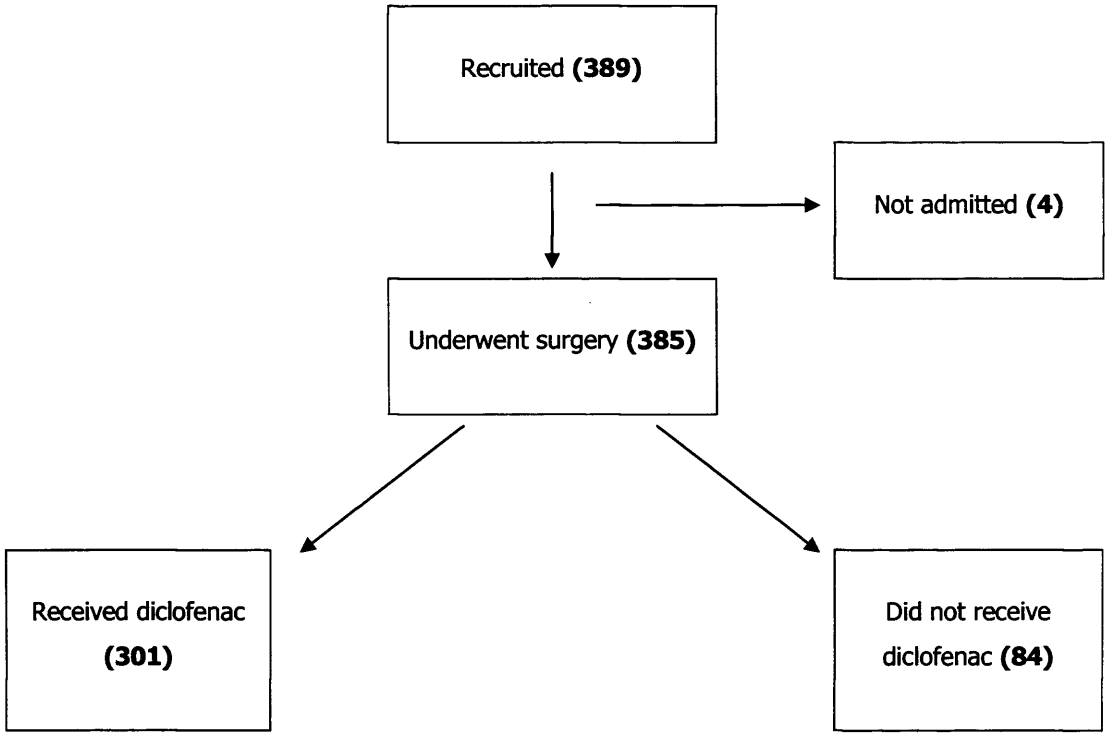


Figure 3.5: Schematic diagram of patient recruitment.

Of the four patients not admitted for surgery, two had their operations at a different hospital due to a waiting list reduction initiative, and two had their operations cancelled, either permanently or at least not rescheduled within the study period.

3.5.2 Demographics and Diclofenac Utilisation

Table 3.1 gives the demographic details for the 385 patients who were recruited and admitted for surgery at one of the study hospitals.

Table 3.1: Demographic details of patients recruited to the adverse event monitoring study.

		Frequency given as number (percentage) or mean (range) as appropriate			
			Received diclofenac		
		All Patients	Yes	No	M-W test
Age (years)		6.0 (0.3-12.9)	6.3 (0.9-12.9)	4.7 (0.3-12.6)	p<0.001
Weight (kg)		22.7 (5.3-80)	23.9 (7.6-80)	18.9 (5.3-54.4)	p<0.001
Stay length (days)		3.1 (1-117)	2.6 (1-26)	4.9 (1-117)	p=0.004
Male		221 (57%)	177 (59%)	44 (52%)	
Female		164 (43%)	124 (41%)	40 (48%)	
No known allergies		314	249 (79%)	65 (21%)	
At least one known allergy		71	52 (73%)	19 (27%)	
No asthma		336	261 (78%)	75 (23%)	χ^2 test
Mild asthma		30	27 (90%)	3 (10%)	p=0.17
Asthma		19	13 (68%)	6 (32%)	
Surgery type:					
	Dental	137	119 (87%)	18 (13%)	
	Ear nose & throat	12	12 (100%)	0 (0%)	
*	General	69	48 (70%)	21 (30%)	
	Orthopaedic	49	44 (90%)	5 (10%)	
	Plastic/craniofacial	56	45 (80%)	11 (20%)	
*	Urology	62	33 (53%)	29 (47%)	

* Certain procedures (hernia repair, circumcision, orchidopexy) carried out by general surgeons and urologists - classification made by surgeon specialty.

In most (89 percent) cases the first dose of diclofenac was given as a suppository in the operating theatre. The majority (69 percent) of post-operative doses were given as a proportion of a soluble tablet, with 33 percent of patients receiving suppositories and 18 percent enteric-coated tablets. The median number of doses per patient was one (range one to 22) with most patients receiving ibuprofen in the post-operative period. The majority of patients received a dose of approximately 1mg/kg, but doses of up to 2mg/kg were used. Twenty-one percent of patients received a 'high dose', which was classified as being intentionally given greater than 1mg/kg. For example, a 17kg child given a 25mg suppository would be classified as receiving a 'high dose' as the nearest whole suppository size to 1mg/kg would be 12.5mg. Similarly, a 20kg child given a 25mg suppository would be classified as receiving a standard dose.

3.5.3 Adverse Events

Adverse event data was collected from 380 patients in total who each received at least one dose of diclofenac. These included 301 patients from the main observational study, eight

patients from the pilot study who received diclofenac, and 71 patients from the pharmacokinetic study (Chapter Two) who all received a single dose of diclofenac suspension. The demographic details of these patients are given in table 3.2, along with the number of adverse events that occurred.

Table 3.2: Demographic details of 380 patients included in analysis of adverse events.

	Frequency given as mean (range) or number (percentage) as appropriate
Age (years)	5.9 (0.9-12.9)
Weight (kg)	22.7 (7.6-80)
Stay length (days)	2.3 (1-26)
Male	225 (59%)
Female	155 (41%)
No known allergies	318 (84%)
At least one known allergy	62 (16%)
No asthma	336 (88%)
Mild asthma	29 (8%)
Asthma	15 (4%)
Did not experience an adverse event	250 (66%)
One adverse event	81 (21%)
Two adverse events	26 (7%)
Three adverse events	9 (2%)
Four adverse events	8 (2%)
Five adverse events	4 (1%)
Six adverse events	2 (1%)

A total of 224 adverse events were recorded in 130 patients. A classification of receiving a 'high dose' had no bearing on the incidence of any adverse events, or on the incidence of adverse events classed as 'possible' or 'probable/likely'. Figure 3.6 gives the number, type and causality assessment classification of all adverse events according to the WHO criteria.

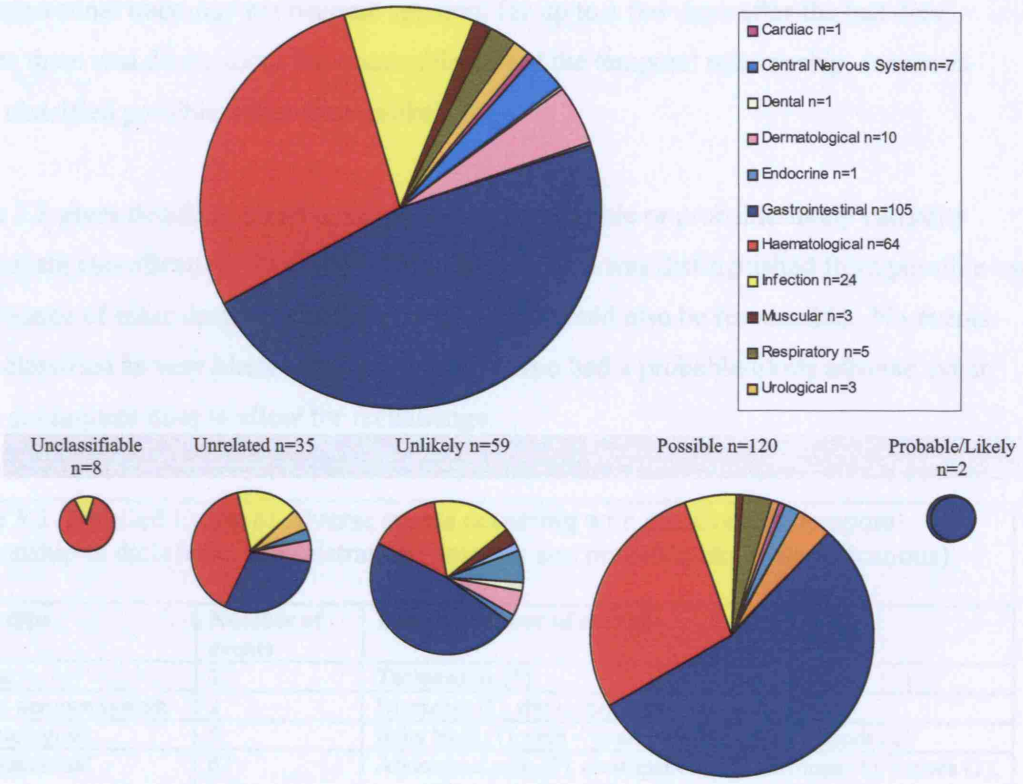


Figure 3.6: Number, type and cause of adverse events experienced by patients receiving diclofenac. Causality was classified by criteria in the WHO definition in figure 3.1.

The unclassifiable adverse events were all laboratory abnormalities found in blood tests taken after the administration of diclofenac where no pre-diclofenac blood tests were available for comparison. These events were therefore unclassifiable as the time of onset was unknown. The unrelated adverse events occurred before diclofenac was administered. Temporal relationship was the key factor in separating the unlikely from the possible classifications. A reasonable temporal relationship would depend on the type of adverse event, and the known pharmacokinetics of diclofenac. For example, bleeding which would be mediated by reversible COX-1 inhibition in platelets would not be expected to last much longer than eight hours after the final dose of diclofenac, as the drug would be almost

entirely eliminated by this time. Other adverse events, such as mucosal damage in the gastrointestinal tract may not become apparent for up to a few days after the last dose. Where there was doubt about the reasonableness of the temporal relationship, reactions were classified possible rather than unlikely.

Table 3.3 gives details of all adverse events with a possible or probable/likely causality assessment classification. Probable/likely classification was distinguished from possible by the absence of other drugs or disease processes that could also be responsible. No events were classified as very likely/certain as no child who had a probable/likely adverse event had a subsequent dose to allow for rechallenge.

Table 3.3: Detailed listing of adverse events occurring with a reasonable temporal relationship to diclofenac administration (possible and probable/likely classifications).

Event type	**Number of events**	**Details (number of events)**
Cardiac	1	Tachycardia (1).
Central nervous system	2	Dizziness (1), drowsiness (1).
Dermatological	5	Itchy back (1), rash – generalised (2), rash – hands (2).
Gastrointestinal	67	Abdominal pain (5), constipation (1), diarrhoea (1), nausea (2), occult blood aspirated from nasogastric tube (1), rectal irritation (1), vomited (56).
Haematological	34	Excessive blood loss from drains (2), excessive ooze from wound (4), laboratory values outside range (26), nosebleed (1), re-admitted to theatre due to bleeding (1).
Infection	7	Bacterial culture from swabs (3), laboratory values outside range (2), pyrexia (2).
Muscular	1	Muscle spasm (1)
Respiratory	4	Hyperventilation (1), laryngospasm (2), wheeziness (1).
Urological	1	Catheter blocked (1).
Total	122	

3.5.4 Probable/Likely Adverse Drug Reactions

Two adverse events were classified as being probable/likely adverse drug reactions caused by diclofenac according to the WHO criteria; details are given in table 3.4.

Table 3.4: Probable/likely diclofenac adverse drug reactions.		
Background	**Adverse event**	**Comments**
(079) Male 7yrs, admitted for excision of multiple naevi on his back.	Developed diarrhoea on the day after the operation. Received a diclofenac suppository in the operating theatre.	Reasonable temporal relationship – operation late afternoon, symptoms early morning - and no other alternative causes were apparent.
(F042) Female 5yrs, admitted for dental extractions.	Complained of rectal irritation post-operatively. Received a diclofenac suppository in the operating theatre.	Good temporal relationship, diclofenac most likely cause.

3.5.6 Serious Adverse Events

Twenty-three adverse events were classified as serious and were reviewed by the expert panel. Five of these were classified as unrelated to diclofenac, three of these five events were intra-operative bleeds requiring blood transfusions, all of which occurred before diclofenac had been administered as a practice of giving diclofenac at the end of long procedures was common.

Table 3.5: Serious adverse events 'unrelated' to diclofenac.		
Background	**Adverse event**	**Comments**
(039) Male 3yrs, admitted for closure of ileostomy.	Problems re-establishing feeding post-operatively leading to prolonged hospital admission and the requirement for total parenteral nutrition.	Diclofenac was not given intra-operatively and was started on day two post-surgery when poor feeding had already been noted. Furthermore, feeding problems continued for over a week after the last diclofenac dose.
(091) Male 1yr, admitted for cranial vault re-modelling.	Massive intra-operative bleed requiring fluid, red blood cell and plasma replacement.	Diclofenac was not given until the evening, after the operation.
(156) Male 4yrs, admitted for cranial vault expansion.	Intra-operative bleed requiring fluid, red blood cell and whole blood replacement.	Diclofenac was not given until the day after the operation.
(188) Female 1yr, admitted for cranial fronto-orbital re-modelling.	Very low haemoglobin (7g/dL) noted in theatre, required overnight blood transfusion.	Onset before diclofenac was given (at the end of the operation).
(P009) Male 11yrs, admitted for bladder neck reconstruction and closure	Intra-operative complications – blood and urine leaking from incision, re explored and sealed hole.	Diclofenac was not given until day three after the operation.

The other two unrelated serious adverse events were a case of poor feeding requiring total parenteral nutrition in a patient who underwent ileostomy closure, the onset of which was before diclofenac was administered, and the final event was a peri-operative complication, again before diclofenac was administered. Details of these events are given in table 3.5. Eleven serious adverse events were classified as unlikely to be diclofenac adverse drug reactions, details are given in table 3.6.

Table 3.6: Serious adverse events 'unlikely' to be diclofenac adverse drug reactions.

Background	Adverse event	Comments
(026) Female 3yrs, admitted for cranial fronto-orbital re-modelling.	Re-hospitalised one week post discharge due to wound infection requiring debridement and intravenous antibiotics.	Received a single dose of diclofenac in theatre. Time of onset of infection unclear but showing no signs of infection on discharge (five days after the dose of diclofenac).
(086) Female 4yrs, admitted for insertion of gastric feeding tube. Type I diabetes.	Loss of glycaemic control prolonged hospitalisation in the post-operative period.	Received a single dose of diclofenac in theatre, problems with blood sugars started 36 hours later and continued until day seven, during which time the patient did not receive any diclofenac.
(096) Male 2yrs, admitted for Nissens fundiplication and gastrostomy formation.	Patient was unable to tolerate feeding for three weeks after the operation, prolonging hospitalisation. Received 16 doses of diclofenac in the first six days post-operatively.	Feeding problems did not resolve for two weeks after the last dose of diclofenac, unlikely that gastric irritation leading to anorexia would be this prolonged. No drops in haemoglobin, red blood cells or any other markers which would suggest a gastrointestinal bleed.
(104) Male 1yr, admitted for cranial vault re-modelling.	Developed rash and pyrexia on day four post-operation, which prolonged hospitalisation. Had four doses of diclofenac, the last one was the day after the operation. Mother was breast feeding and started a course of flucloxacillin on day three post-operation. Cultures of *Staph. aureus* were grown from femoral line samples. Diclofenac re-started on day six post-operation with no recurrence of rash.	The temporal relationship to the onset of the rash and pyrexia was poor, and rechallenge with diclofenac did not cause a rash/pyrexia. This reaction was thought to be probably a combination of femoral line infection and possible flucloxacillin allergy from breast milk.
(141) Male 10yrs, admitted for posterior spinal fusion.	Wound did not heal and continued to ooze for eight days, prolonging hospitalisation. Received seven doses of diclofenac in the first three days post-operatively. Cultured *Staph. epidermidis* from wound swabs and clinical impression was wound infection.	Poor temporal relationship as wound continued to ooze after diclofenac stopped. Diclofenac inhibition of COX-1 is reversible so would expect platelet aggregation to normalise on withdrawal. No clotting times measured, but wound infection provides compelling causative factor.

Table 3.6 (continued).

(165) Male 7yrs, admitted for Nissens fundiplication. History of recurrent respiratory tract infections.	Suffered a collapsed lung and pneumonia on the day after the operation, requiring a week-long stay in the intensive care unit. Received a single dose of diclofenac in theatre.	Respiratory complications liable to be either COX-1 mediated or allergic-type reactions, neither of which have a temporal association which would be likely to extend to the day after a single dose of diclofenac. Patient's medical history provides more compelling contributing factor.
(172) Female 1yr, admitted for removal of cystic hygroma.	Admitted to local hospital six days post discharge with wound infection.	Received a single dose of diclofenac in theatre. Time of onset of infection unclear but showing no signs of infection on discharge (two days after the dose of diclofenac).
(F037) Male 11yrs, admitted for circumcision.	Developed a wound infection one week after discharge, which required treatment with oral antibiotics. Received a single dose of diclofenac in the operating theatre.	Poor temporal relationship, onset was a week after diclofenac dosing.
(F064) Male 4yrs, admitted for circumcision.	Developed a wound infection three days after discharge, which cleared with oral antibiotics. Received a single dose of diclofenac in the operating theatre.	Poor temporal relationship, onset was three days after diclofenac dosing.
(F069) Male 9yrs, admitted for dental extractions.	Developed a throat infection four days after discharge, which cleared with oral antibiotics. Received a single dose of diclofenac in the operating theatre.	Poor temporal relationship, onset was four days after diclofenac dosing.
(F079) Male 9yrs, admitted for dental extractions	Developed a throat infection three days after discharge, which cleared with oral antibiotics prescribed by dentist. Received a single dose of diclofenac in the operating theatre.	Poor temporal relationship, onset was three days after diclofenac dosing.

Seven serious adverse events were classified as possibly diclofenac adverse drug reactions. Details are given in table 3.7.

Table 3.7: Serious adverse events 'possibly' diclofenac adverse drug reactions.

Background	Adverse event	Comments
(037) Male 3yrs, admitted for excision of neavus on his back.	Had to return to theatre on day one post-operation to re-stitch the wound as it re-opened. Diclofenac given in theatre on both occasions.	Temporal relationship is reasonable although no excessive oozing was noted on rechallenge with diclofenac. Furthermore the likely cause was that the patient was very active and probably excessive movement caused the stitches to separate.
(105) Female 2yrs, admitted for Nissens fundiplication, history of laryngomalacia and on regular nebulised salbutamol at home.	Day three post-operation developed polyphonic wheeze, treated with salbutamol, ipratropium and monteleukast and prolonged hospitalisation. Received eight doses of diclofenac between day one and day three.	Reasonable temporal relationship for COX-1 mediated narrowing of airways. However, patient was on regular nebulised salbutamol at home which was omitted after the operation due to an oversight. This provides an alternative plausible cause.
(143) Female 7yrs, admitted for spinal fusion.	Wound swab taken on day three post operation isolated a coagulase negative Staph. Flucloxacillin restarted and hospitalisation prolonged. Received nine doses of diclofenac during the first four days.	Reasonable temporal relationship with diclofenac administration, but contamination either in theatre or on the ward provides a more plausible explanation.
(190) Male 11yrs, admitted for revision of craniofacial re-modelling.	Increased drainage of blood noted on the evening of the operation from the drains inserted, required blood transfusion. Received a single dose of diclofenac in theatre, APTT elevated post-operatively but no pre-operative samples available.	Temporal relationship reasonable but unknown whether APTT was elevated pre-operatively. Also depletion of clotting factors and small quantities of heparin used to keep lines patent may affect APTT.
(F019) Male 1yr, admitted for hypospadias repair.	Had an intra-operative laryngospasm, oxygen saturation dropped to 40% and developed bradycardia during the operation, required oxygen and brief cardiac massage. Received a dose of diclofenac at the start of the operation.	There is a reasonable temporal relationship with diclofenac administration but the patient also received propofol and fentanyl with a reasonable temporal relationship and was also intubated, which is the most likely cause as the patient showed no sign of allergic-type reaction.
(F096) Male 3yrs, admitted for hypospadias repair.	Vomited several times after the operation despite use of anti-emetic (cyclizine) and prolonged hospitalisation for a day. Received a single dose of diclofenac in the operating theatre.	There is a reasonable temporal relationship with the onset of vomiting and diclofenac administration. The patient also received several other drugs, which provide other possible causes.
(F098) Male 9yrs, admitted for hypospadias repair.	Urinary catheter would not drain so patient had to be re-admitted to theatre the following day for insertion of a suprapubic catheter under general anaesthetic. Received diclofenac on both occasions.	Reasonable temporal relationship but other possible causes include a blockage in the catheter or it becoming dislodged/misplaced.

3.6 Discussion

Not all patients recruited to the study received diclofenac. Whilst the primary aim of this study was not to investigate diclofenac utilisation, prospective recruitment of patients has provided some insight, as shown in table 3.1. Patients who received diclofenac were significantly older and stayed for shorter times in hospital than those that did not. This suggests a tendency for prescribers to avoid using diclofenac in younger children, possibly due to worries about renal function maturation (Kearns GL et al, 2003), and in patients who stay for longer and are therefore possibly undergoing more complex surgery. It may also be the case that patients who stay in hospital longer have more other underlying conditions such as renal dysfunction or propensity for gastrointestinal bleeding, meaning that diclofenac is not prescribed.

The most interesting finding of the utilisation analysis is that there was no significant difference in the frequency of diclofenac prescribing in asthmatic children. Because of the difficulty in diagnosing asthma in infants and young children, patients were split between 'mild asthma', meaning that asthma was mentioned in the past medical history and/or was being treated with bronchodilators only (step one), and asthma requiring at least regular inhaled steroid treatment (step two or above) (*BTS Guideline*, 2004). Despite the possibility of NSAID-induced bronchospasm, it would appear that the prescribers in this study were generally unconcerned about the use of NSAIDs in asthmatic children. This is probably due to a combination of factors. Firstly a large randomised trial in febrile children where 1879 asthmatics received ibuprofen or paracetamol, resulted in a paradoxical significant decrease in asthma morbidity in the ibuprofen group (Lesko SM et al, 2002). Secondly, as diclofenac is an effective opiate-sparing analgesic agent, and as the first dose is being given in a hospital, it is likely that most prescribers judge the potential benefit to the patient greater than the risk of harm due to bronchospasm.

Most patients (66 percent) did not experience any adverse events, and many of the adverse events recorded were clearly unrelated, or unlikely to be related to diclofenac due to their time of onset in relation to dosing. There were 122 adverse events that occurred within a reasonable time relating to diclofenac administration, and were therefore classified as possible or probable/likely adverse events.

The only cardiovascular adverse event with a reasonable temporal relationship was tachycardia reported in the nursing notes when the patient returned from the operating theatre after a laporoscopic orchidopexy. The heart rate was 197 beats in the immediate post-operative period, and reduced to 116 beats per minute six hours later. Whilst this event had a reasonable temporal relationship with diclofenac administration, amongst other agents administered during the operation the patient also received atracurium. Atracurium competes with acetyl-choline at the neuromuscular junction and can cause tachycardia either through histamine release or direct decrease in blood pressure with rebound increases in heart rate (*Martindale*, 2002). This provides a more plausible cause than diclofenac, which is not known to increase heart rate.

Two adverse events affecting the central nervous system were recorded that had a reasonable temporal relationship with diclofenac. Both dizziness and drowsiness are reported to be adverse effects of diclofenac (*Diclofenac SPC*, 2002), although in both of these cases the patients were also given opiate analgesia (fentanyl and morphine) and propofol for anaesthesia, which provide more plausible alternative causes. However, as both of these events are known adverse drug reactions associated with diclofenac, and if it was assumed that both were caused by diclofenac, this gives an incidence (95 percent confidence interval) of minor central nervous system disturbance of 2/380 (0.06 to 1.9 percent); the term minor being used to signify a non-serious adverse drug reaction.

Five dermatological adverse events had a temporal relationship making them possibly related to diclofenac administration. Two of these were rashes on the hands, where topical anaesthetic creams had been used so although the temporal relationship coincided with diclofenac administration, the localisation of the reaction suggests that topical anaesthetics were the more likely cause. One patient developed an erythemous rash on his face, torso and legs approximately five hours after taking a pre-operative dose of diclofenac suspension. This patient was discharged as he was otherwise well, and on telephone questioning it was discovered that the following day his general practitioner had diagnosed tonsillitis, which provides an alternative cause along with the opiate and other medications he received during the admission. A second patient developed a macular patchy rash on

each limb within 30 minutes of diclofenac administration which resolved approximately one hour later. This patient also received a dose of paracetamol at the same time as the diclofenac suspension, providing another potential causative factor and making the causality possible. The final dermatological adverse event with a reasonable temporal association was a patient who complained of an itchy back on day three of regular diclofenac treatment for post-operative pain. The itching resolved with two doses of chlorpheniramine and did not reoccur despite diclofenac continuing until day five. The onset of this event had a reasonable temporal relationship with diclofenac but the patient was also treated with other antibiotics and opiate analgesics on the day of the operation, which could also have been causative.

The benefit to cost/time ratio of determining whether any of the previous three reactions were diclofenac adverse reactions are relatively dubious as none of the reactions were serious and NSAIDs from a different chemical class such as ibuprofen could be used in future. Fixed drug eruptions to diclofenac have been previously noted and can present as non-pigmenting erythema or priuritus (Mahboob A & Haroon TS, 1998) and occur in the same place upon re-challenge. Re-challenge might be the best way to determine whether these two reactions were caused by diclofenac, and whether they were fixed drug eruption-types. The rash that occurred within half an hour of dosing may be a mild allergic-type effect due to its rapid temporal onset, although lack of other histamine-mediated symptoms would suggest otherwise (Riedl MA & Casillas AM, 2003). If diclofenac were an antibiotic or from some other class of drug which may be required for life-saving treatment in the future, then it would be necessary to investigate these patients further possibly by taking blood samples for radioallergosorbent testing (RAST), which certainly may be pertinent in the case of urticaria and the rash with the 30 minute onset which could possibly be allergic reactions mediated by immunoglobulin E (IgE). The problem with RAST is that diclofenac metabolites would also need to be acquired in case any of these are causative, and NSAIDs often form immune complexes not mediated through IgE giving a potential for false negatives (Riedl MA & Casillas AM, 2003). As NSAIDs are a chemically heterogeneous class of drugs so avoidance of diclofenac would not necessitate avoidance of ibuprofen for example, and their use is not potentially life-saving, re-challenge will not be undertaken in these patients. Without rechallenge data and therefore assuming that

diclofenac may have been causative in the three systemic cases (itchy back, erythematous rash and macular patchy rash) then the incidence (95 percent confidence interval) of non-serious dermatological reaction caused by diclofenac would be 3/380 (0.016 to 2.3 percent).

The largest group of adverse events within a reasonable temporal relationship to diclofenac were related to the gastrointestinal tract. Five patients complained of abdominal pain, which as a relatively non-specific symptom may or may not be related to gastrointestinal pathology. In all of these cases, the patients had recently undergone gastrointestinal or urological surgery, which is a more compelling cause for the adverse event than gastrointestinal damage due to diclofenac, as there were no other symptoms. There is an argument to say that unexpected therapeutic failure could be classified as a (type F) adverse drug reaction (Edwards IR & Aronson JK, 2000), but as not every patient can be expected to receive complete analgesia from diclofenac (McQuay HJ & Moore RA, 1998) these events could not be called adverse drug reactions using such a classification.

One patient was found to have occult blood present in the stomach on aspiration from the nasogastric tube on day two of treatment with diclofenac after a Nissens fundiplication, an operation to treat gastro-oesophageal reflux by wrapping the stomach around the fundus to cause closure to the oesophagus on filling of the stomach. Aspirates were clear of blood by day four, but reoccurred on day eight after a large feed, four days after diclofenac had stopped. The amount of blood loss was probably quite small as no drop in haemoglobin or red blood cells was seen on full blood counts. The patient's gastric surgery is probably more likely to have caused this event rather than bleeding due to diclofenac.

Two patients suffered nausea without vomiting with a reasonable time after receiving diclofenac, however both of these patients also received opiate analgesia. In total 56 patients vomited within a reasonable time of diclofenac administration, but all had also received at least one other drug which is known to cause vomiting, in all cases either an opiate, anaesthetic agent, or both. In most of these cases patients received another more emetogenic drug than diclofenac, as randomised controlled trials comparing NSAIDs and opiates have repeatedly shown decreased incidence of nausea and vomiting in the NSAID

group (Cardwell M et al, 2005). Attributing causality of any of these cases of nausea and vomiting to a specific drug is unfeasible with the present study design.

The two adverse events classified as probable/likely to be diclofenac adverse drug reactions were both gastro-intestinal effects. One patient complained of rectal irritation and one of diarrhoea in the post-operative period after receiving a diclofenac suppository in the operating theatre. Rectal irritation is a known adverse effect of diclofenac, as a weak acid it could be mediated by direct action on the rectal mucosa or simply mechanical irritation caused by suppository insertion. In either case, this is a probable adverse drug reaction as it is a reaction to the medicine as a whole. The case of diarrhoea is less straight-forward but still likely to be caused by diclofenac in the absence of any other known possible causes. This patient experienced a single episode of diarrhoea on the morning following surgery in which he received a diclofenac suppository. Diarrhoea is a known adverse effect of diclofenac and no other potential causes were obvious (no other medications received are known to cause diarrhoea, other family members were not affected). It could be argued that on the day of an operation children have a different diet, in that they are starved pre-operatively, and may eat more or different foods (possibly as a treat) after the operation but in this instance there was no indication that this was the case. In total 287 patients received at least one diclofenac suppository, making the incidence (95 percent confidence interval) of rectal irritation 1/287 (0.009 to 1.9 percent). The incidence (95 percent confidence interval) of diarrhoea associated with diclofenac in this study is 1/380 (0.007 to 1.5 percent).

The majority of haematological adverse events with a reasonable temporal onset with diclofenac administration were laboratory tests outside the reference range. Most of these were decreases in haemoglobin, red blood cell count, and haematocrit indicating blood loss, and almost certainly due to surgery. Nine patients had elevated activated partial thromboplastin times (APTT) compared with baseline pre-operative values, suggesting lower coagulability in the intrinsic coagulation pathway. The incidence of these adverse events is relatively high as only 121 patients underwent routine full blood counts. Furthermore, as no noticeable harm came to the patient or treatment was necessary, the significance of these results is questionable. Of the nine patients with elevated clotting

times from laboratory values, seven had undergone craniofacial vault remodelling and the other two had undergone spinal fusions. Procedures in the craniofacial region have a high risk of bleeding complications due to a process known as disseminated intravascular coagulation. Disseminated intravascular coagulation often occurs after cranial surgery as the brain cortex contains high thromboplastin levels which increase in response to trauma and cause extensive clotting in the microcirculation. Consequently clotting factors become depleted which can result in elevated APTT and bleeding (Heeson M et al, 1997). Spinal fusion surgery is also a major operation with a high degree of potential blood loss and in these circumstances, depletion of clotting factors is a more likely explanation than diclofenac-induced bleeding, given that none of the 112 other patients, some of whom had more minor procedures, had elevations in laboratory clotting times with a reasonable temporal relationship to diclofenac, suggests that the procedure type may be of more significance.

Of the four patients with subjectively excessive wound ooze, two were dental patients having had multiple teeth removed, one had a revision of cleft palate scar and the final patient had an alveolar bone graft. No pathology results were available for these patients but again they were procedures in the craniofacial area where bleeding is a known problem (Heeson M et al, 1997). The patient who had a nosebleed after dental surgery reported often having nose bleeds, again no pathology laboratory data was available. Finally a patient had to be taken back to the operating theatre for wound re-stitching due to bleeding, but had been very active after the operation and pulled out the stitches due to excessive movement. It would therefore seem that bleeding complications are more likely to be due to surgery type and factors such as excessive movement in the post-operative period rather than diclofenac administration. This is highlighted in table 3.8, showing that out of all patients prescribed diclofenac, the majority who had bleeding complications with a reasonable temporal relationship to diclofenac administration had undergone procedures with a high intrinsic risk of bleeding.

Table 3.8: Number of patients having at least one bleeding adverse events including elevated laboratory clotting times with a reasonable temporal relationship to diclofenac administration against surgery type.

Surgery type	**Number of patients prescribed diclofenac.**	**Number having a bleeding adverse event with a reasonable temporal relationship to diclofenac administration (percentage).**	**Procedure undergone in patients experiencing bleeding/wound ooze/excessive loss from drains.**
Craniofacial	23	9 (39%)	Cranial remodelling.
Dental	119	3 (3%)	Dental extractions.
Ear nose and throat	12	1 (8%)	Alveolar bone graft.
General	48	0	
Orthopaedic	45	2 (4%)	Spinal fusion.
Plastic	80	1 (1%)	Naevus excision – pulled stitches.
Urology	53	0	
Total	380	16 (4%)	

In a systematic literature review on bleeding in paediatric patients undergoing tonsillectomy, 955 children were randomised to receive either a NSAID or placebo/non-NSAID analgesic (Cardwell M et al, 2005). There was no significant increase in bleeding events suggesting that the bleeding events seen in the present study are unlikely to be attributable to diclofenac, despite their reasonable temporal relationship.

Seven patients had signs of infection with a reasonable temporal relationship to diclofenac administration. No mechanism from diclofenac's known pharmacology, such as immunosuppression, would make patients more susceptible to infection. The only potential explanation could be that elevated bleeding time may allow for infection to develop, and indeed four of these reactions did accompany subjectively long bleeding times or elevated clotting times reported by the pathology laboratories, but as discussed earlier, it is unlikely that diclofenac does in fact alter bleeding time.

One patient suffered muscle spasm within a reasonable time of diclofenac administration. However, this is not a known adverse effect of diclofenac and the patient had undergone a bilateral soft tissue release in the hips, a procedure for which muscle spasm is a common complication.

Four respiratory adverse events had a reasonable temporal onset compared with diclofenac administration, none of which were allergic-type bronchospasms. Hyperventilation was recorded in the nursing notes for one patient who became extremely distressed after an operation for multiple dental extractions. The cause of this adverse event was therefore most likely to be emotional rather than pharmacological. Two patients experienced laryngospasm in the operating theatre, and although the temporal relationship was reasonable with diclofenac administration, the events coincided more closely with intubation, which is the most likely cause.

The final respiratory adverse event was wheezing in a patient with pre-existing laryngomalacia who was on regular nebulised salbutamol at home, which had been mistakenly omitted in the post-operative period. The onset of wheezing progressively worsened over the three days after the operation during which the patient was also on regular diclofenac for post-operative pain. Diclofenac was stopped as it was no longer required (it was not thought to be causative by the respiratory physician who reviewed the patient) and wheezing continued for five days after. The only certain way to check if this patient did suffer wheezing as a result of diclofenac and not because of the omitted nebulised salbutamol would be to rechallenge them with a bronchoprovocation test (Szczeklik A & Stevenson DD, 2003). Whilst the increased wheezing did occur after diclofenac administration, gradually worsening over three days, NSAID-induced bronchospasm usually occurs within three hours and is often accompanied by rhinitis, conjunctival injection (bloodshot eyes) and flushing in the head and neck region (Szczeklik A & Stevenson DD, 2003), none of which the patient experienced meaning that this reaction was probably not diclofenac-induced.

This study included forty asthmatic children who did not experience bronchospasm with diclofenac. In addition, a bronchoprovocation challenge in 70 asthmatic children given diclofenac also did not find any who suffered bronchospasm (Short JA et al, 2000). This gives a total of 110 asthmatic children in whom diclofenac did not induce bronchospasm, making the maximum incidence of diclofenac-induced bronchospasm 2.7 percent. NSAID-induced bronchospasm in asthmatics is thought to occur in approximately 11 percent of asthmatics and its mechanism is probably through COX-1 mediated leukotriene production

(Szczeklik A & Stevenson DD, 2003). As diclofenac is a more potent inhibitor of COX-2 than COX-1 (Cryer B & Feldman M, 1998), this may explain the lower incidence of bronchospasm seen here.

The final possible adverse drug reaction was urological. The urological event was a urinary catheter that would not drain and required re-positioning and prolonged hospitalisation. Whilst the temporal relationship between the catheter problems and diclofenac administration in theatre were reasonable, the problems did not re-occur when the catheter was replaced even though diclofenac was also given on this occasion. The temporal relationship made it a possible adverse drug reaction, but no pharmacological reason can be envisaged.

No patients had elevations in serum creatinine or urea in the post-operative period but this is possibly a reflection of the fact that very few (23) had samples taken for urea and electrolytes after the operation. In adults, a transient asymptomatic reduction in creatinine clearance occurred in the post-operative period when comparing NSAIDs with placebo (Lee A et al, 2004). Although in the present study no symptomatic reductions in renal function occurred, it was hoped that more patients would have undergone testing for urea and creatininie so that comparisons between ages could have been made. In retrospect, it would have been very interesting to have routinely measured these parameters, but no research nurse was available for this study and it would have constituted an intervention, so sampling for clinical reasons had to be relied upon.

The majority of these adverse events classified as possible adverse drug reactions were relatively minor in that they were not classified as serious. In the peri-operative period multiple factors including other medications and surgical interventions can provide alternative, equally plausible causes, and so despite the use of causality assessment, reasoned judgements still need to be made. Making these judgements is difficult, especially as baseline data that would be provided by a placebo group is unavailable using the present study design. In addition to looking at placebo groups, it would be interesting to know the proportion of healthy children taking no medications who have a minor adverse event each day. The incidence of 'adverse non-drug reactions' in children would be a

useful, if difficult to obtain, baseline from which observational studies such as this could use as a comparator. Two such studies have been conducted in adults using questionnaires given to subjects taking no medications (Meyer FP et al, 1996, Reidenberg MM & Lowenthal DT, 1968). An interesting finding of these studies is that only 19 and 11 percent of subjects respectively reported no adverse non-drug reactions when given a list of potential effects such as drowsiness, nausea, vomiting, bleeding of gums on brushing teeth, urticaria and rash. Whilst these minor adverse effects are often important from the perspective of the patient or parent, the only definitive way to ascertain their true incidence of reactions caused by a medicine would be in a rigorous, randomised, placebo controlled blinded study on a single operative procedure.

Other possible study designs include a controlled trial. A placebo controlled study with diclofenac which is known to be efficacious is clearly unethical as the placebo group would be denied pain relief, and a comparative study against another NSAID such as ibuprofen would give some information on the relative merits of different NSAIDs, but the resources necessary to recruit sufficient numbers of patients to ensure that all common adverse reactions were seen (300 per group), would be difficult to justify.

This study has revealed no cases of allergic-type bronchospasm, acute renal failure, serious gastrointestinal bleeding, or hepatotoxicity. This means the incidence of these serious adverse drug reactions caused by diclofenac is less than 0.8 percent (3/380) in children with a confidence of 95 percent (Hanley JA & Lippman-Hand A, 1983). The incidence of these reactions in adults is also less than 0.8 percent (Catalano MA, 1986).

A weakness of this study is that not all patients admitted for surgery during the recruitment phase were entered leaving the possibility of some form of selection bias. The main reason that not all patients were entered was the need for written informed consent as patient and parent questioning was undertaken, both during hospital admission, and by telephone follow-up. Prospective recruitment was deemed necessary to minimise the effect of subject and investigator bias, and also allowed for an investigation into diclofenac utilisation. Recruiting patients prior to surgery often proved difficult, as many were admitted late in the evening or on the morning of the operation. In this period before surgery patients need to

have routine observations and be admitted by the ward nurse, clerked by a junior surgical doctor, have consent for the procedure taken by a senior surgical doctor, and be assessed for their fitness for anaesthesia by an anaesthetist. It is probably this that accounts for the relatively low face-to-face recruitment rate of 62 percent; the prospect of entering a research study at a relatively stressful time may not be very appealing. Some parents who refused to take part may have misunderstood that the study was observational, probably due to the four-side information sheet required by the ethics committee (Appendix 7.7) and stress involved with their child's impending operation. Whilst the pre-admission clinic proved to be a less stressful environment in which to recruit patients, not all patients were scheduled to attend one. Postal recruitment rates were poor (21 percent) possibly because the letters were usually sent well in advance of admission, meaning that some parents with the intention to participate may understandably forget to return the forms.

In order to improve the efficiency of this study in terms of the proportion of patients recruited, retrospective medical notes review could have been used. The disadvantages with this method would have been the potential for investigator bias and the difficulty in interpreting adverse events purely from the written record. The ability to confirm adverse event details with both ward staff and parents, in addition to being able to check the time of dosing, for example on occasions in the anaesthetic record where it was difficult to tell whether diclofenac had been given at the start or end of the procedure, probably meant that the method used was superior to a retrospective notes review.

3.7 Conclusions

In terms of non-serious diclofenac adverse drug reactions, it would appear that similar types occur in adults and children. These are reactions such as minor dermatological effects, minor gastrointestinal effects such as rectal irritation with suppositories, diarrhoea, and minor central nervous system disturbances such as dizziness and drowsiness. A wider question as to whether these reactions are actually drug-induced, or whether in fact some are 'adverse non-drug reactions' could not be answered with this observational study design. Indeed, to confirm any of the possible adverse drug reactions seen in this study, re-challenge would be required, and this is inappropriate for a drug such as diclofenac. The

incidence of nausea and vomiting caused by diclofenac could not be assessed in this study due to the concomitant use of opiates and anaesthetic agents in all patients.

The second main finding of this study is that no serious adverse events that could readily be recognised as known diclofenac adverse drug reactions were seen. This study took place in tertiary and secondary care settings, and included a range of procedures and diclofenac formulations. As most children in the UK undergo operations in similar settings, then these results should be transferable to the general paediatric population. This means that there is a 95 percent probability that serious adverse drug reactions such as acute renal failure, symptomatic gastrointestinal bleeding, bronchospasm and hepatotoxicity have an incidence of less than 0.8 percent in paediatric patients treated with diclofenac in the peri-operative period. In addition, the incidence of diclofenac-induced bronchospasm in asthmatic children is less than 2.7 percent.

The final conclusion is that an observational study conducted in the peri-operative period, where several medicines and other interventions are given simultaneously, makes attributing the cause of non-specific adverse events to a single drug very difficult. However, the proven efficacy of diclofenac precluded a placebo controlled trial from being conducted, and comparison with another NSAID that is commonly used for acute pain such as ibuprofen or ketorolac would have been difficult to blind due to the differing formulations available, and difficult to conduct due to the need for standardisation of other factors such as operative procedure and type and dose of other drugs. This would also only have allowed a comparison between NSAIDs and would not be able to delineate class specific adverse effects. The problems encountered in this study highlight the need for common adverse effects to be ascertained in early phase clinical trials, which often have a placebo group or are in patients taking no other medications, before the drug achieves widespread usage. When children are treated with medicines licensed for adults, it is often difficult to ascertain the propensity for adverse effects in observational studies in clinical practice. However, this study has demonstrated a low incidence of common adverse events that can be clearly associated with diclofenac therapy.

Chapter FOUR: Incidence of Rare Adverse Drug Reactions to Diclofenac for Peri-Operative Pain in Children

4.1 Introduction

The previous chapter investigated common adverse drug reactions to diclofenac, and in finding that none were serious, concluded the incidence of such reactions to be less than 0.8 percent. Although a small number of adverse events were attributed to diclofenac, the lack of a control group meant that it was not possible to make useful estimates as to the propensity of diclofenac to cause non-serious yet significant (especially to patients and their parents) events such as nausea and vomiting, or to completely discount diclofenac as contributing to cases of excessive bleeding, despite there being other more plausible explanations. The frequency of diclofenac-induced bronchospasm in asthmatic children also requires investigation to determine whether it should be withheld in these patients.

In order to better assess the incidence of serious adverse drug reactions to diclofenac, it will be necessary to investigate a larger population of paediatric patients being treated for acute pain than was available in the previous study. One possible way in to achieve this would be through the use of epidemiological drug-utilisation databases such as the General Practice Research Database (Wood L & Martinez C, 2004). Such databases routinely collect patient information entered by the general practitioner and can be a very powerful tool in assessing the type and prevalence of adverse drug reactions in children (Wong ICK & Murray ML, 2005). The problem with using such databases is that despite large numbers of paediatric patients being available for study, they do not directly cover secondary care, relying on the general practitioner to enter information given by the hospital. It is unlikely therefore that the database would contain information about peri-operative doses of diclofenac: although the general practitioner would hopefully be informed if a patient had a serious adverse reaction to diclofenac whilst in hospital, no reliable estimate could be made of incidence due to a lack of denominator (number of children given diclofenac in hospital).

As diclofenac is mainly used for acute peri-operative pain in children, it is necessary to study its use in this setting. Whilst no large databases of routine clinical care in hospitalised children are available, the outcomes of thousands of paediatric patients treated

for acute pain are available in published studies. Systematic literature review of studies where children are given diclofenac for acute pain, including but not limited to the peri-operative period, migraine, renal colic and soft tissue injury or fractures, provides a useable source of information for estimating the incidence of serious adverse drug reactions, as such reactions should be reported, and a number of children given diclofenac (denominator) is available to calculate incidence.

It must be noted that reporting of adverse events is generally poor in randomised trials (Ioannidis JPA & Lau J, 2001), and lack of a standard methodology for reporting adverse events, means there is little consistency in the way they are reported (Nuovo J & Sather C, 2007). Deriving an estimate of the incidence of non-serious adverse drug reactions using studies where the aim was solely to investigate efficacy is therefore not possible. However, where a serious adverse event occurs, it is reasonable to expect this will be reported, or if not, the patient will probably be excluded from the final analysis. Writing to authors of studies where dropouts are not adequately accounted for, or where any ambiguity remains from the published report, should overcome this problem provided they are able to produce the raw data. In addition, where serious adverse events are concerned, a wider variety of study designs can be used. These may include observational studies, pharmacokinetic studies, case series, or any other published report in which children are given diclofenac for acute pain. Combining these studies will give a number of patients exposed to act as a denominator for the number of serious adverse reactions seen.

In addition to investigating the incidence of serious adverse reactions, a systematic review will provide an opportunity to ascertain the prevalence of less well-defined effects such as nausea and vomiting, and bleeding for which a comparator group is required to ascertain the propensity of the test drug to cause the effect. Whilst reporting of such effects is often poor (Ioannidis JPA & Lau J, 2001), it may be possible to identify well-conducted randomised controlled studies with diclofenac as one of the treatment arms and with adequate (numerical) reporting of adverse events within each group. Combining the results of these studies may allow a comparison of effects such as nausea and vomiting, and bleeding times with either placebo or other analgesics.

It is possible that serious adverse events caused by diclofenac are so rare that none will be seen with relatively large combined numbers of exposed patients. For this reason, an analysis of case reports and spontaneous reports will also be undertaken in order to investigate whether similar types of serious adverse reactions to diclofenac occur in children and adults. Unlike most systematic reviews and meta-analyses, which rarely assess therapeutic safety (Ernst E & Pittler MH, 2005), this review will be a form of teleoanalysis (Loke YK et al, 2004, Wald NJ & Morris JK, 2003) in that instead of only including a single study design, for example randomised controlled trials in meta-analysis, a combination of study designs will be used to investigate diclofenac adverse drug reactions in children. In addition to data from published literature being included, a summary of cases reported to the Medicines and Healthcare Products Regulatory Agency Yellow Card scheme will be included. The Yellow Card scheme is a system of adverse reaction reporting by health professionals and patients where suspected adverse reactions are reported to the regulator via a standardised form (printed on yellow card) (Pirmohammed M et al, 1998).

By utilising the full range of published study types, especially with the inclusion of case reports and case series (Aronson JK, 2005), it is hoped that if patterns of adverse drug reactions to diclofenac are different between children and adults, that these may become apparent.

4.1.1 Identification of Previous Relevant Reviews

Before conducting a systematic literature review it is important to check whether the topic in question has already been covered. The following search was undertaken to identify previous systematic reviews on diclofenac for acute pain in children:

Cochrane Library: The keyword "diclofenac" was used to search the whole library with 60 of 3440 systematic reviews/protocols and 27 of 4645 from the Database of Abstracts of Reviews of Effects (DARE) retrieved. Of these, three completed reviews on diclofenac use in acute pain (Barden J et al, 2004, Lee A et al, 2000, Holdgate A & Pollock T, 2004) were found, none of which included children. One review on bleeding complications of NSAIDs in paediatric tonsillectomy was found (Cardwell M et al, 2005). This review overlaps

somewhat with the proposed review although it only studies paediatric tonsillectomy patients and includes any NSAID, not just diclofenac. One relevant abstract from DARE was a review of pharmacologic interventions in paediatric pain (Maikler VE, 1998), but this contained no analgesic efficacy or safety data specifically on diclofenac.

National Research Register: Keyword searches on diclofenac and NSAIDs. No completed or on-going reviews on diclofenac for acute pain in children were found.

Medline: Table 4.1 shows the standardised search strategy (*CRD Guide*, 2001) used to identify reviews in Medline. In addition, a keyword search on diclofenac limited to review articles and children was undertaken.

Table 4.1: Medline search filter to identify reviews on diclofenac (*CRD Guide*, 2001).

Step	Keyword	Number of hits
1	(systematic adj review$)	5176
2	(data adj synthesis)	3137
3	(published adj studies)	4273
4	(data adj extraction)	2842
5	meta-analysis	5313
6	meta-analysis.ti.	4729
7	comment	247089
8	letter	502001
9	editorial	160056
10	animal	3587999
11	human	8414382
12	10 not (10 and 11)	2766922
13	diclofenac	3091
14	13 not (7 or 8 or 9 or 12)	2168
15	or/1-6	20437
16	14 and 15	9
17	from 16 keep 1, 3, 5-7	5
$ = Truncation Adj = Adjacent to		

Three potentially relevant reviews were found, all of which focused on NSAIDs in general. The first looked at haemorrhage after tonsillectomy (Krishna S et al, 2003) although only Medline and the Science Citation Index was searched and limits to the English language means the review could not be classed as systematic. Three diclofenac studies were included and the review's focus was on haemorrhage so no efficacy measures were

included. The meta-analysis showed that the 285 patients who received non-aspirin NSAIDs did not have significant increases in post-operative bleeding. Adults were included although most patients were children of varying age and the authors acknowledge that further prospective studies are needed to determine the relationship between NSAIDs and post tonsillectomy bleeding. The second study identified was a systematic review of post-operative analgesia and vomiting in day-case surgery (McQuay HJ & Moore RA, 1998). This comprehensive systematic review showed diclofenac 50mg to be as effective as intramuscular morphine 10mg in adults, but did not include paediatric patients. The third review on NSAID analgesic efficacy and bleeding in children (Romsing J & Walther-Larsen S, 1997) was not systematic in its search strategy, using only Medline limited to English language papers. Although the results were not combined, the authors summarised the findings of nine studies, mainly in children, showing diclofenac to decrease pain scores (three out of seven studies), opioid requirements (four out of seven studies) and paracetamol requirements (four out of five studies) without significant increases in post-operative bleeding (five out of five studies). The only adverse event looked for was bleeding.

Embase: A keyword search on diclofenac limited to review articles and children was undertaken. Only one further review was found comparing NSAID efficacy with paracetamol in children (Anderson BJ, 2004). No search strategy was given so it was not a systematic literature review and the main focus was on methods used to compare NSAIDs and paracetamol.

International Pharmaceutical Abstracts: The keyword "diclofenac" was used. Three further reviews were identified which looked at NSAID use in children (Kokki H, 2003, Litalien C & Jacqz-Aigrain E, 2001, Peters JW et al, 1999), although none were systematic reviews of clinical trials.

Cinahl: Diclofenac limited to children was searched. No further reviews were found.

Whilst this search was not completely exhaustive, it is unlikely that there are any published systematic reviews on the safety of diclofenac for acute pain in children that investigate

multiple procedure types and attempt to ascertain the incidence of serious adverse drug reactions.

4.2 Aims

To assess the comparative incidence of non-serious adverse reactions to diclofenac such as nausea, vomiting, mild skin rash and bleeding.

To investigate the types of serious adverse drug reactions experienced by children treated with diclofenac for acute pain.

To assess the incidence of rare serious adverse drug reactions caused by diclofenac when used for acute pain in children.

4.3 Objectives

Conduct a systematic literature review to identify:

- Controlled studies comparing diclofenac with either placebo or other analgesics, which specifically investigate and report adverse events.
- Any published reports detailing a serious adverse drug reaction caused by diclofenac.
- Published studies reporting diclofenac use for acute pain in children that include reporting of adverse events.

4.4 Method

The search criteria were designed to include any study type where systemic (intravenous, intramuscular, rectal, oral) diclofenac was used for acute pain. Acute pain was defined as any painful condition requiring a limited course of diclofenac treatment and included but was not limited to: peri-operative pain, migraine, renal colic, soft tissue injuries and fractures. To be eligible for analysis, study participants must have received at least one dose of diclofenac and be aged 18 years or younger. In addition, numbers of adverse events, or a statement saying that there were none, was required, and authors were contacted in cases where this was ambiguous. Figure 4.1 gives the databases searched for the review.

1. Embase
2. Medline
3. Cinahl
4. Cochrane Library
5. Pascal (French)
6. Lilacs (South American)
7. Sigle (grey literature)
8. Dissertation abstracts
9. ISI (conference abstracts)
10. National Research Register
11. Current Controlled Trials
12. Clinicaltrials.gov
13. IPA (International Pharmaceutical Abstracts)
14. Pharmline
15. Biosis
16. Pharmaceutical companies marketing diclofenac 25mg tablets or 12.5/25mg suppositories in the UK approached for unpublished data in children.
17. Hand search of Paediatric Anaesthesia (1991 - 1994) – period not indexed in Medline.
18. References within included studies.

Figure 4.1: Data sources searched to retrieve papers on diclofenac for acute pain in children.

Data sources were searched individually and where multiple search terms were possible, the search strategy general principle was:

[Diclofenac text word OR Diclofenac and related subject headings OR Brand names]

AND

[Terms for pain OR Pain and management OR Cause/type of pain text words OR Cause/type of pain subject headings OR Treatments for pain text word and subject heading]

AND

Children.

The Embase search strategy is given in table 4.2.

Table 4.2: Embase search strategy prepared after brainstorming session by the review group.

Step	Search term
1	Diclofenac$ (textword)
2	Diclofenac (subject heading) or diclofenac-potassium (subject heading) or diclofenac-diethylamine (subject heading) or diclofenac colestyramine (subject heading)
3	Voltarol$ (textword) or Voltaren$ (textword) or Diclomax (textword) or Motifene (textword)
4	1 or 2 or 3
5	(Explode) pain (subject heading)
6	(Explode) neuralgia (subject heading)
7	(Explode) nociception (subject heading)
8	(Pain or neuralgia or nociception or nociperception) and (manag$ or control$ or relief or relieve$) (textwords)
9	Injur$ (textword) or fracture (textword) or headache (textword) or migraine (textword) or cephalalgiA (textword) or hemicraniA (textword) or neuralgi$ (textword) or hyperalgesi$ (textword) or earache (textword) or toothache (textword) or colic (textword)
10	Postoperative-pain (subject heading) or kidney-colic (subject heading) or migraine (subject heading) or soft-tissue-injury (subject heading)
11	Analgesi$ (textword) or antinocicept$ (textword)
12	(Explode) analgesia (subject heading)
13	(Explode) antinociception (subject heading)
14	Postoperative-analgesia (subject heading)
15	5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16	Child (textword) or adolescent (textword) or infant (textword) or paediatric (textword)
17	Child (subject heading) or infant (subject heading) or baby (subject heading) or preschool-child (subject heading) or school-child (subject heading) or adolescent (subject heading)
18	16 or 17
19	4 and 15 and 18
$ = Truncation Adj = Adjacent to	

A preliminary screen of extracted study titles and abstracts was carried out to remove any which obviously did not fit the inclusion criteria of studying children treated with diclofenac for acute pain. A second detailed screen was performed on the remaining studies by obtaining the full article. Where there was ambiguity over any necessary information the authors were contacted by post or e-mail. Data on number of patients given diclofenac, diclofenac dose, whether any patients were asthmatic, whether there were any serious adverse events were collated in SPSS (Version 13). Controlled studies that looked for adverse events and compared diclofenac with another treatment were further assessed for quality of blinding, and numbers of adverse events occurring between groups was recorded.

All studies were assigned a measure of quality for adverse event reporting, which aimed to assess the risk that adverse events were missed or did occur but were not reported. The classifications can be found in table 4.3, with studies having to meet each standard within a category to be classified as low, moderate or high.

Table 4.3: Quality assessment criteria for included safety studies.

Quality:	**High**	**Moderate**	**Low**
Study design.	Prospective, number of patients exposed to diclofenac given.	Prospective, number of patients exposed to diclofenac given.	Prospective or retrospective, number of patients exposed to diclofenac given.
Adverse event monitoring.	Evidence of monitoring for adverse events.	Evidence of monitoring for adverse events.	Spontaneous reporting of adverse events.
Adverse event reporting.	Specific adverse events reported dropouts accounted for.	Adverse events may be grouped by system, dropouts accounted for.	Adverse events may be grouped by system, dropouts not accounted for.
Assessment of causality linking adverse events and adverse drug reactions.	Use of causality algorithm or difference versus placebo in double-blind RCT.	Causality may be attributed by clinical judgement or difference versus placebo in double-blind RCT.	Causality may be attributed by clinical judgement or difference versus placebo in double-blind RCT.
Follow-up.	Post-discharge follow-up on at least one occasion.	No evidence of follow-up.	No evidence of follow-up.

RCT = Randomised Controlled Trial.

For both rounds of screening and for the quality assessment, two reviewers (the second being Dr Imogen Savage) independently assessed papers and then met to agree upon inclusions, exclusions and classification. Any adverse event reported by the author to be serious was recorded. Where adverse events were reported but no indication of seriousness was provided, the same definition as the observational study (Chapter Three) was used, with such an event being: fatal, life-threatening, prolonging hospitalisation, causing persistent or significant disability or incapacity, or requiring medical or surgical intervention to prevent any of these. The search yielded some qualitative data in the form of case reports where a denominator was not present. These were included in the qualitative part of the review along with an analysis of the UK Committee for Safety of Medicines spontaneous reporting 'Yellow Card' scheme, a special search of which was

requested to provide details of any report of an adverse drug reaction caused by diclofenac in a patients aged 18 years or less.

The 95 percent confidence intervals for incidence of events were calculated with the statistical package STATA (version 9) assuming a Poisson distribution. Peto odds ratios for nausea and/or vomiting were calculated using the systematic review package Review Manager (version 4.2).

4.5 Results

4.5.1 Search Results

Screening of 825 papers from the initial search, the hand-search of Paediatric Anaesthesia (1991-1995 when not indexed by Medline), and checking references of included studies gave 106 papers for final analysis (Appendix 7.10). On detailed screening, 62 were included, characteristics of these studies is given in table 4.4.

Table 4.4: Characteristics of included studies (Appendix 7.10).

Study	**Andrzejowski 2002.**
Methods	Randomised controlled trial.
Participants	133 children (ASA 1-2) undergoing dental surgery.
Interventions	1. Swab soaked in bupivacaine 0.25% (60). 2. Swab soaked in saline (60). All patients received rectal diclofenac 1mg/kg.
Outcomes	1. Pain. 2. Bleeding.
Notes	13 dropouts due to inability to self-score pain. Wrote to authors: all these patients received diclofenac and none had any serious adverse events. Adverse events monitored for, no serious adverse events occurred in the 133 receiving diclofenac. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Azuma 1982.**
Methods	Comparative trial of analgesia, diclofenac pharmacokinetics. Not randomised or blinded.
Participants	40 children post-tonsillectomy.
Interventions	1. Rectal diclofenac 1mg/kg (20). 2. Non-NSAID group (20).
Outcomes	1. Analgesia. 2. Pharmacokinetics. 3. Adverse events.
Notes	Not enough information to include in comparative study. Adverse events monitored for, no serious adverse events occurred in the 20 receiving diclofenac. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Baer 1992.**
Methods	Randomised controlled trial, investigator blind.
Participants	44 children (ASA 1) undergoing adenoidectomy.
Interventions	1. Rectal diclofenac 12.5mg (19). 2. Rectal paracetamol 125mg (25).
Outcomes	1. Pain. 2. Bleeding. 3. Behaviour. 4. Post-operative complications.
Notes	Adverse events monitored for, no adverse events in either group and no serious adverse events. Asthmatics: excluded. Safety quality: moderate.
Allocation concealment	Unclear.
Study	**Berry 1992.**
Methods	Randomised controlled trial, investigator blind.
Participants	40 children (ASA 1-2) undergoing adenotonsillectomy.
Interventions	1. Intravenous diclofenac 1mg/kg (20). 2. Intravenous papaveretum 0.2mg/kg (20).
Outcomes	1. Pain. 2. Bleeding. 3. Vomiting. 4. Venous irritation.
Notes	Wrote to author of letter on venous sequelae of diclofenac in children which mentioned a clinical study – directed to paper in The Journal of the Pain Society. Blinding centralised with hospital pharmacy prepared doses. Papaveretum and paracetamol rescue analgesia used. Adverse events monitored for, 20 children had diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate
Study	**Bone 1998.**
Methods	Randomised controlled trial, investigator blind.
Participants	60 children undergoing tonsillectomy.
Interventions	1. Rectal diclofenac 2mg/kg (20). 2. Intramuscular papaveretum 0.2mg/kg (20). 3. No treatment (20).
Outcomes	1. Pain. 2. Nausea and vomiting. 3. Respiratory rate. 4. Restlessness.
Notes	Paracetamol and papaveretum rescue analgesia used. Risk of bias as no placebo injection/suppositories used. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Elhakim 2003.**
Methods	Randomised controlled trial.
Participants	50 children (ASA 1-2) undergoing tonsillectomy.
Interventions	1. Intramuscular ketamine 0.1mg/kg (25). 2. Intramuscular saline, equivalent volume to ketamine dose (25). All children received rectal diclofenac 2mg/kg.
Outcomes	1. Pain. 2. Adverse events.
Notes	Evidence of monitoring for adverse events, no serious adverse events reported. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Engelhardt 2001.**
Methods	Randomised controlled trial.
Participants	29 children undergoing adenotonsillectomy.
Interventions	1. Sublingual morphine 0.1mg/kg (14). 2. Intravenous morphine 0.1mg/kg (15). All patients received rectal diclofenac 1mg/kg.
Outcomes	1. Pain. 2. Sedation. 3. Adverse events.
Notes	Adverse events monitored for, 29 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Engelhardt 2003.**
Methods	Randomised controlled trial.
Participants	60 children undergoing tonsillectomy +/- adenoidectomy.
Interventions	1. Intravenous morphine 0.1mg/kg (20). 2. Intravenous tramadol 1mg/kg (20). 3. Intravenous tramadol 2mg/kg (20). All (60) received rectal diclofenac 1mg/kg.
Outcomes	1. Pain. 2. Adverse events until discharge.
Notes	Adverse events monitored for, 60 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Filatov 2000.**
Methods	Randomised controlled trial.
Participants	126 children (ASA 1) undergoing adenoidectomy.
Interventions	1.Rectal diclofenac 12.5mg, rectal diazepam 0.5mg/kg, intravenous glycopyrrolate 5μg/kg, topical EMLA® to hand (20). 2. Rectal diclofenac 12.5mg, rectal diazepam 0.5mg/kg topical EMLA® to hand (21). 3. Oral ketamine in cola drink 6mg/kg, intravenous glycopyrrolate 5μg/kg (30). 4. Oral ketamine in cola drink 6mg/kg (29). Placebo local anaesthetic cream, suppository, rectal fluid, oral cola drink and injection used.
Outcomes	1. Pain. 2. Adverse events.
Notes	Wrote to authors as 26 patients excluded causing risk of bias, no reply therefore cannot include in comparitive analysis. Adverse events monitored for, 41 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: Low
Allocation concealment	Unclear.
Study	**Findlow1997.**
Methods	Randomised controlled trial.
Participants	40 boys undergoing orchidopexy.
Interventions	1. Caudal bupivacaine 0.25% 0.5mL/kg and ketamine 0.5mg/kg. (20). 2. Ilioinguinal block bupivacaine 0.25% 0.5mL/kg (20). All (40) received rectal diclofenac 1-2mg/kg.
Outcomes	1. Pain. 2. Adverse events including follow-up.
Notes	Drop-outs: two did not undergo surgery or receive diclofenac, two no follow-up was possible but received diclofenac. Adverse events monitored for, 38 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Fischer 1992.**
Methods	Randomised controlled trial.
Participants	60 children undergoing orthopaedic surgery.
Interventions	1. Oral diclofenac 50mg twice or three times daily (50). 2. Oral serrapeptase 5mg 1-2 tablets three times daily (50).
Outcomes	1. Pain. 2. Swelling. 3. Adverse events.
Notes	Cannot use for comparative analysis – five serrapeptase and three diclofenac drop-outs, unaccounted for and no blinding. Adverse events monitored for, 47 patients received diclofenac, one event rated by patient as severe but unclear which one from table, could be: vomiting, headache, stomach pain or erythema. Asthmatics: unknown. Safety quality: Low.
Allocation concealment	Inadequate

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Gadiyar 1995.**
Methods	Randomised controlled trial, investigator blinded.
Participants	39 children (ASA 1 or 2) undergoing day case surgery.
Interventions	1. Rectal diclofenac 1mg/kg plus caudal bupivacaine 0.125% plus adrenaline 1:400 000 1mL/kg (19). 2. Caudal bupivacaine 0.125% plus adrenaline 1:400 000 1mL/kg only (20).
Outcomes	1. Pain. 2. Sedation. 3. Time to passing urine. 4. Time to eating/drinking. 5. Excessive bleeding.
Notes	Paracetamol rescue analgesia used. Adverse events monitored for including post-discharge follow-up. No mention of nausea and vomiting but subjectively looked at bleeding, no increase in the diclofenac group. No reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Gananca 1991.**
Methods	Randomised controlled trial.
Participants	58 children with pharyngo-tonsillitis.
Interventions	1. Oral diclofenac resinate 0.5mg/kg (29). 2. Oral nimesulide 5mg/kg (29). Seven day treatment, all patients also received oral amoxicillin 30mg/kg/day.
Outcomes	1. Pain. 2. Adverse events. 3. Taste of medication.
Notes	Include in comparative study but open study with no blinding so risk of bias. Specifically mentions no other medicines used during treatment course, adverse events monitored for, 29 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate
Study	**Hanafy 2004.**
Methods	Randomised controlled trial.
Participants	51 children (ASA 1-2) undergoing adenotonsillectomy.
Interventions	1. Intravenous dexmedetomidine 0.5µg/kg (23). 2. Intravenous saline (23). All patients received rectal diclofenac 1mg/kg.
Outcomes	1. Post-operative agitation. 2. Adverse events.
Notes	Wrote to authors (as 5 patients excluded - risk of bias) no response. Adverse events monitored for, 46 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: Low.
Allocation concealment	Not applicable.
Study	**Holder 1997.**
Methods	Randomised controlled trial.
Participants	45 boys (ASA 1-2) undergoing circumcision.
Interventions	1. Subcutaneous ring block with bupivacaine 0.25% 2mg/kg (16). 2. Subpubic penile block using bupivacaine 0.5% 0.2mL/kg (24). Seven patients received diclofenac 1mg/kg rescue analgesia.
Outcomes	1. Pain. 2. Adverse events.
Notes	Adverse events monitored for, seven patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Homer 2002.**
Methods	Audit.
Participants	100 children undergoing tonsillectomy.
Interventions	77 received diclofenac.
Outcomes	1. Pain. 2. Haemorrhage rate.
Notes	Retrospective audit, adverse events monitored for, 77 patients received diclofenac, no reports of serious adverse events (mentions one patient with reactionary haemorrhage settled with conservative treatment). Asthmatics: unknown. Safety quality: low.
Allocation concealment	Not applicable.
Study	**Hultcrantz 2004.**
Methods	Randomised controlled trial.
Participants	150 children undergoing tonsillectomy.
Interventions	1. Radiofrequency technique (49). 2. Standard technique (43). All children received diclofenac (route not stated) 0.7-1mg/kg/dose.
Outcomes	1. Pain. 2. Adverse events including follow-up.
Notes	Only 92 of the 150 randomised were operated on. Five were excluded for not accepting randomisation, five operated on without being randomised and the rest had surgery cancelled. Adverse events monitored for, 92 patients received diclofenac, no reports of serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Kokinsky 1999.**
Methods	Audit.
Participants	200 children undergoing day case surgery.
Interventions	68 children received rectal diclofenac 0.8-2mg/kg intra-operatively, and 10 post-operatively
Outcomes	1. Pain. 2. Adverse events.
Notes	Wrote to authors for clarification on adverse events. Two patients who received diclofenac had serious adverse events (one protracted vomiting requiring prolonged admission, one re-operated due to bleeding in penile surgery). Asthmatics: unknown. Safety quality: low.
Allocation concealment	Not applicable.
Study	**Korpela 1990.**
Methods	Pharmacokinetic study.
Participants	10 children undergoing minor surgery.
Interventions	1. Intravenous diclofenac 0.5mg/kg infused over five minutes (5). 2. Intravenous diclofenac 0.5mg/kg infused over 15 minutes (5).
Outcomes	1. Pharmacokinetics. 2. Adverse events.
Notes	Adverse events monitored for and contacted authors, 10 patients received diclofenac, no serious adverse events. Asthmatics: excluded. Safety quality: low.
Allocation concealment	Not applicable.
Study	**Kurokawa 2002.**
Methods	Case series.
Participants	Girls undergoing laparoscopic nephroureterectomy.
Interventions	Laparoscopic nephroureterectomy, two received rectal diclofenac 12.5mg.
Outcomes	1. Post-operative complications. 2. Pain.
Notes	Adverse events monitored for, two patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: low.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Lai 2005.**
Methods	Case report.
Participants	7 year old girl with sore throat and fever.
Interventions	Intramuscular diclofenac 30mg.
Outcomes	Developed aseptic tissue necrosis at the injection site.
Notes	Include in qualitative review. Patient required surgical debridement and primary closure.
Allocation concealment	Not applicable.
Study	**Lambert 2000.**
Methods	Comparative study.
Participants	19 children and 23 adults undergoing appendicectomy.
Interventions	Standardised analgesia including diclofenac 1.5mg/kg in children.
Outcomes	1. Pain measured by PCA morphine consumprion. 2. Adverse events.
Notes	Some evidence of monitoring for adverse events, contacted author for confirmation, no serious adverse events occurred in the paediatric patients. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Lenotev 2004.**
Methods	Comparative study.
Participants	57 children undergoing minor surgery.
Interventions	1. Rectal or intramuscular diclofenac 1mg/kg (47). 2. Analgin and Promedol (10).
Outcomes	1. Pain. 2. Adverse events.
Notes	Some evidence of monitoring for adverse events, no serious adverse events in 47 patients receiving diclofenac. Asthmatics: unknown. Safety quality: low.
Allocation concealment	Unclear.
Study	**Littlejohn 1996.**
Methods	Randomised controlled trial, investigator blind.
Participants	60 children undergoing day case dental extractions.
Interventions	1. Rectal diclofenac 1-2mg/kg (19). 2. Intravenous nalbuphine 0.3mg/kg (21). 3. No analgesia (20).
Outcomes	1. Pain. 2. Subjective bleeding. 3. Time to waking. 4. Nausea.
Notes	Paracetamol rescue analgesia. Adverse events monitored for and no serious adverse events. Asthmatics: excluded. Safety quality: moderate, no evidence of follow-up.
Allocation concealment	Adequate.
Study	**Mak 2001.**
Methods	Randomised controlled trial, investigator blind.
Participants	187 boys undergoing circumscision.
Interventions	1. Rectal diclofenac 1mg/kg and intravenous fentanyl 0.5μg/kg (61). 2. Dorsal penile nerve block with 0.5% bupivacaine (63). 3. Caudal block with bupivacaine 0.5mL/kg (61).
Outcomes	1. Pain. 2. Adverse events.
Notes	Cannot use in comparative section as comparison was diclofenac plus fentanyl (one patient in each group suffered bleeding, three in each of the local block groups vomited versus one in diclofenac/fentanyl group). Drop-outs in caudal group due to inability to perform caudal block. Adverse events monitored for, 61 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Martindale 2004.**
Methods	Randomised controlled trial.
Participants	60 children undergoing day case surgery.
Interventions	1. Caudal bupivacaine 0.25% 1mL/kg (20). 2. Caudal bupivacaine 0.25% 1mL/kg plus S(+)-ketamine 0.5mg/kg (20). 3. Caudal bupivacaine 0.25% 1mL/kg plus intravenous S(+)-ketamine 0.5mg/kg. All patients received rectal diclofenac 1mg/kg.
Outcomes	1. Pain. 2. Adverse events.
Notes	One patient was withdrawn - did not have surgery so 59 received diclofenac. Adverse events monitored for, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**McGowan 1998.**
Methods	Randomised controlled trial.
Participants	61 boys (ASA 1-2) undergoing circumcision.
Interventions	1. Rectal diclofenac 2-2.5mg/kg plus penile block with bupivacaine 0.5% 0.3mL/kg (20). 2. Penile block with bupivacaine 0.5% 0.3mL/kg (18). 3. Rectal diclofenac 2-2.5mg/kg (20).
Outcomes	1. Pain. 2. Bleeding. 3. Nausea and vomiting.
Notes	Got raw data. Include the diclofenac plus penile block vs penile block in the comparative analysis. Three dropouts – penile block failed. Adverse events monitored for and two diclofenac patients had serious adverse events, both late haemorrhage requiring overnight hospitalisation and reoperation in one case. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Unclear.
Study	**Mendham 1996.**
Methods	Randomised controlled trial, investigator blind.
Participants	127 children undergoing tonsillectomy or adenotonsillectomy.
Interventions	1. Rectal diclofenac 1mg/kg (25). 2. Rectal diclofenac 1mg/kg plus intravenous fentanyl 0.75μg/kg (33). 3. Intravenous tenoxicam 0.4mg/kg (35). 4. Intravenous tenoxicam 0.4mg/kg plus intravenous fentanyl 0.75μg/kg (28).
Outcomes	1. Pain. 2. Bleeding. 3. Sedation.
Notes	Wrote to author for more detail on exclusions and drop-outs. Six patients excluded – two from group 1, one from group 2 and two from group 3 returned to theatre with post-operative bleeding, one from group 2 was excluded due to insufficient data. Morphine rescue analgesia used. Adverse events monitored for, 62 patients received diclofenac, three serious adverse events. Asthmatics: excluded severe. Safety quality: Moderate.
Allocation concealment	Adequate.
Study	**Menezes 2001.**
Methods	Comparative study.
Participants	100 children (ASA 1-3) undergoing orthopaedic surgery.
Interventions	1. Rectal diclofenac 1mg/kg (20). 2. Caudal bupivacaine 0.25% plus adrenaline (1:400000) 0.5-1mL/kg (20). 3. Caudal fentanyl 1.5μg/kg (20). 4. Caudal morphine 30μg/kg (20). 5. Caudal sulfentanil 0.3μg/kg (20).
Outcomes	1. Pain. 2. Adverse events.
Notes	Randomisation by order of admission and no blinding. Some patients recived midazolam pre-medication but cannot tell who - exclude from comparative analysis. Adverse events monitored for, 20 patients received diclofenac with no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Mikawa 1995.**
Methods	Randomised placebo controlled trial.
Participants	140 children (ASA 1) undergoing strabismus surgery.
Interventions	1. Diazepam 0.4mg/kg (35). 2. Clonidine 2μg/kg (35). 3. Clonidine 4μg/kg (35) all dissolved in apple juice. 4. Placebo (35). All patients received rectal diclofenac 12.5 or 25mg.
Outcomes	1. Vomiting. 2. Adverse events.
Notes	Adverse events monitored for, 140 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Moores 1990.**
Methods	Randomised controlled trial, investigator blinded.
Participants	43 children undergoing day case herniotomy.
Interventions	1. Rectal diclofenac 2.5mg/kg (20). 2. Caudal bupivacaine 0.25% 1mL/kg (18).
Outcomes	1. Pain. 2. Vomiting. 3. Oral intake. 4. Mobility. 5. Passing urine. 6. Sleep disturbance.
Notes	Paracetamol rescue analgesia used. Wrote to authors, no response. Five dropouts, three incomplete questionnaires, one discharged early, one surgical complications caused overnight admission, original allocation unclear. Blinding maintained by placing gauze over sacral hiatus for all patients. Asthmatics: unknown. Safety quality: Low.
Allocation concealment	Adequate.
Study	**Morton 1999.**
Methods	Randomised controlled trial.
Participants	80 children undergoing appendicectomy.
Interventions	1. Rectal diclofenac 1mg/kg 8 hourly (20). 2. Rectal paracetamol 20mg/kg loading dose than 15mg/kg 6 hourly (20). 3. Diclofenac plus paracetamol in the doses above (20). 4. No simple analgesic (20). All patients on patient controlled morphine infusions.
Outcomes	1. Pain. 2. Adverse events.
Notes	No information on blinding. Differential morphine consumption used as efficacy measure so cannot compare adverse events between groups. Adverse events monitored for, 40 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.
Study	**Mukherjee 2001.**
Methods	Randomised controlled trial.
Participants	60 children (ASA 1-2) undergoing tonsillectomy or adenotonsillectomy.
Interventions	1. Intramuscular morphine 100μg/kg (27). 2. Intravenous fentanyl 1μg/kg (29). All patients received rectal diclofenac 1-1.5mg/kg.
Outcomes	1. Pain. 2. Adverse events.
Notes	Wrote to authors authors, four drop-outs did receive diclofenac and did not suffer any serious adverse events. Adverse events monitored for, 60 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Murphy 2000.**
Methods	Pharmacokinetic study.
Participants	20 children undergoing adenotonsillectomy.
Interventions	Rectal diclofenac 2mg/kg.
Outcomes	1. Pharmacokinetics. 2. Adverse events.
Notes	Conference abstract, little detail but does mention no patients suffered any adverse effects. Asthmatics: unknown. Safety quality: low.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Nakayama 1982.**
Methods	Observational study.
Participants	40 children undergoing tonsillectomy.
Interventions	Rectal diclofenac 25mg.
Outcomes	1. Pain. 2. Adverse events.
Notes	Adverse events monitored for, 40 patients received diclofenac, no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Nikolic 2001.**
Methods	Case series.
Participants	28 children admitted with acute tubulointerstitial nephritis.
Interventions	None.
Outcomes	1. Description of treatment and outcome
Notes	One case was attributed to diclofenac, no details on length of treatment given. Child made a full recovery with supportive therapy.
Allocation concealment	Not applicable.
Study	**Nishina 2000.**
Methods	Randomised controlled trial, investigator blind.
Participants	125 children (ASA 1) undergoing ophthalmological surgery.
Interventions	1. Rectal diclofenac 2mg/kg (25). 2. Intravenous flurbiprofen 1mg/kg (25). 3. Oral clonidine 4μg/kg (25). 4. Rectal diclofenac plus oral clonidine (25). 5. Intravenous flurbiprofen plus oral clonidine (25). Placebos used for pre-medication where patient was awake.
Outcomes	1. Pain. 2. Vomiting. 3. Time to eye opening. 4. NSAID complications (rash, priuritus, bronchospasm, convulsions).
Notes	Paracetamol and diclofenac rescue analgesia used. NSAID complications looked for. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Oztekin 2002.**
Methods	Randomised controlled trial, investigator blind.
Participants	40 children (ASA 1-2) undergoing tonsillectomy.
Interventions	1. Rectal diclofenac 1mg/kg (20). 2. No treatment (20).
Outcomes	1. Pain. 2. Adverse events.
Notes	Morphine as rescue analgesia. Adverse events monitored for, 20 patients received diclofenac, one serious adverse event from each group required nasal packaging and prolonged hospitalisation. Asthmatics: excluded. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Robinson 1994.**
Methods	Case note review.
Participants	366 patients undergoing tonsillectomy.
Interventions	Cases of post-tonsillectomy haemorrhage reviewed, not all received diclofenac.
Outcomes	Four children identified to have had post-tonsillectomy haemorrhage, three received diclofenac.
Notes	Include in qualitative review - authors state that they believe the incidence of post-operative bleeding increases with diclofenac use and these cases were likely to be caused by diclofenac.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Romsing 2000.**
Methods	Randomised controlled trial, investigator blind.
Participants	52 children undergoing tonsillectomy.
Interventions	1. Oral diclofenac 2-3mg/kg/24 hours (24). 2. Paracetamol 90mg/kg/24 hours (24). Placebo tablets and oral liquid used to maintain blinding.
Outcomes	1. Pain. 2. Bleeding. 3. Nausea and vomiting.
Notes	Four patients (two from each group) excluded as unwilling to take oral analgesics – no risk of bias as patients did not receive study medication. Asthmatics: excluded. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Ryhanen 1994.**
Methods	Randomised controlled trial, investigator blind.
Participants	299 children (ASA 1) undergoing herniotomy or orchidopexy.
Interventions	1. Intramuscular diclofenac 1mg/kg (70). 2. No analgesia (73). 3. Caudal bupivacaine 0.25% 1mL/kg (57). 4. Caudal bupivacaine 0.25% with adrenaline 5μg/mL 1mL/kg (50).
Outcomes	1. Pain. 2. Adverse events. 3. Diclofenac pharmacokinetics.
Notes	Wrote to author, original data destroyed. 49 drop-outs cannot account for all but author states no patients had serious adverse events. Cannot compare post-operative complication rates as no numbers given (overall percentages). Gauze placed over sacral hiatus to maintain blinding. Adverse events monitored for, 70 patients received diclofenac with no serious adverse events. Asthmatics: excluded. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Sahjananda 2003.**
Methods	Case report.
Participants	Eight year old boy with Kartagener's syndrome.
Interventions	Lobectomy, received diclofenac for acute pain.
Outcomes	Post-operative complications.
Notes	Description of anaesthesia in difficult case. Received diclofenac, no serious adverse events. Asthmatic: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Samarkandi 2005.**
Methods	Randomised controlled trial, investigator blind.
Participants	92 children (ASA 1) undergoing herniotomy.
Interventions	1. Rectal diclofenac 1mg/kg (32). 2. Caudal bupivacaine 0.25% 0.75mL/kg (30). 3. Combination of the above (30).
Outcomes	1. Pain. 2. Vomiting. 3. Urinary retention. 4. Time to ambulation. 5. Sleep on night after operation.
Notes	Pethidine and paracetamol rescue analgesia used. Gauze covering sacral hiatus used for all patients to maintain blinding. Adverse events monitored for, 63 patients received diclofenac with no serious adverse events. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Schiffmann 2005.**
Methods	Case report.
Participants	16 year-old girl two weeks post wisdom teeth removal.
Interventions	Oral diclofenac 75mg twice daily for five days and clindamycin 300mg three times daily for 10 days.
Outcomes	Colon perforation causing peritonitis and requiring peritoneal lavage and colostomy formation, closed after three months with no further complications.
Notes	Include in qualitative review. Diclofenac implicated as lesions typical of NSAID-associated damage and showed no signs of pseudomembrenous colitis possibly induced by clindamycin.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Seaton 2000.**
Methods	Case report.
Participants	15 year old boy with meningococcaemia.
Interventions	Received diclofenac for arthralgia.
Outcomes	Developed widespread limb purpura, protracted immunological phenomena and late-onset gastrointestinal vasculitis.
Notes	Include as case report but note causality uncertain. Contacted author, unsure as to the most likely cause for gastrointestinal pathology – either diclofenac or meningococcal related arthropathy.
Allocation concealment	Not applicable.
Study	**Selin 2004.**
Methods	Comparative study.
Participants	86 children undergoing orthopaedic surgery.
Interventions	1. Intramuscular diclofenac 50-75mg (50). 2. No additional treatment (36).
Outcomes	1. Pain. 2. Bleeding.
Notes	No information on randomisation or blinding. Some evidence adverse events monitored for, 50 patients received diclofenac with no serious adverse events. Asthmatics: unknown. Safety quality: low.
Allocation concealment	Unclear.
Study	**Sen 2001.**
Methods	Case report.
Participants	Nine year-old girl hospitalised for transient synovitis.
Interventions	Discharge medication: oral diclofenac 25mg twice daily.
Outcomes	On the first dose at home patient developed body aches, rash, itching, cutaneous flushing and became febrile. A second dose was administered two hours later (to treat these symptoms) and developed generalised rash and choking. Prompt hospitalisation with haemodynamic and respiratory support, but resuscitation failed.
Notes	Include in qualitative review.
Allocation concealment	Not applicable.
Study	**Swanepoel 1999.**
Methods	Randomised trial, investigator blinded.
Participants	80 children undergoing tonsillectomy.
Interventions	1. Rectal diclofenac 1mg/kg on induction of anaesthesia (40). 2. Oral diclofenac suspension 1mg/kg two hours before surgery (40).
Outcomes	1. Pain.
Notes	Letter describing study, unclear whether adverse events looked for but states all patients discharged home the following day and no problems with haemostasis occurred. Wrote to author who confirmed no serious adverse events occurred. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Syladis 1998.**
Methods	Prospective observational study.
Participants	20 children undergoing cleft palate repair.
Interventions	Rectal diclofenac 1mg/kg in theatre and further post-operative doses if required.
Outcomes	1. Post-operative complications.
Notes	Two non-serious adverse events noted, neither attributed to diclofenac. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Tawalbeh 2001.**
Methods	Randomised controlled trial.
Participants	80 children undergoing tonsillectomy.
Interventions	1. Rectal diclofenac 0.5-1.5mg/kg/dose (41). 2. Oral paracetamol 10-15mg/kg (39).
Outcomes	1. Pain. 2. Prolonged hospitalisation. 3. Bleeding requiring surgical intervention. 4. Nausea and vomiting.
Notes	Uses rescue analgesia. Risk of bias as treatment allocation known. Two serious adverse events (re-hospitalisation) one in each group. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.
Study	**Tay 2002.**
Methods	Randomised controlled trial, investigator blind.
Participants	63 children (ASA 1-2) undergoing bilateral myringotomy.
Interventions	1. Oral diclofenac resinate 0.5mg/kg (30). 2. Oral paracetamol 15mg/kg (33).
Outcomes	1. Pain. 2. Vomiting.
Notes	Fentanyl rescue analgesia used, some evidence of adverse event monitoring, no serious adverse events reported. Asthmatics: unknown. Safety quality: low.
Allocation concealment	Unclear.
Study	**Tewary 1993.**
Methods	Retrospective case record review.
Participants	363 children undergoing tonsillectomy.
Interventions	All patients received rectal diclofenac 25mg on induction of anaesthesia.
Outcomes	1. Reactionary haemorrhage. 2. Return to hospital due to bleeding.
Notes	Six patients either had prolonged hospitalisation or were rehospitalised within 24 hours for reactionary haemorrhage. Five patients returned to hospital with bleeding between days two and eight (11 serious adverse events in total). Asthmatics: unknown. Safety quality: low.
Allocation concealment	Not applicable.
Study	**Thiagarajan 1993.**
Methods	Randomised controlled trial, investigator and anaesthetist blinded.
Participants	198 children (ASA 1-2) undergoing tonsillectomy.
Interventions	1. Intramuscular diclofenac 1mg/kg (91). 2. Intramuscular papaveretum 0.2mg/kg (92).
Outcomes	1. Pain. 2. Bleeding requiring surgical intervention. 3. Subjective blood loss. 4. Nausea and vomiting.
Notes	15 withdrawals, 10 did not receive the study drug and record sheets of five were incomplete. Wrote to authors – none of these five excluded patients had a serious adverse event, but unclear whether they received diclofenac. Can use recovery area adverse events for comparative analysis without rescue analgesia. Evidence of monitoring for adverse events, 91 patients definitely received diclofenac, one serious adverse event – bleeding requiring re-operation. Asthmatics: excluded. Safety quality: moderate.
Allocation concealment	Adequate.
Study	**Van Der Marel 2004.**
Methods	Pharmacokinetic study
Participants	26 children undergoing tonsillectomy.
Interventions	Rectal diclofenac 2mg/kg followed by 1mg/kg eitht hourly.
Outcomes	1. Pharmacokinetics.
Notes	Wrote to authors, no serious adverse events. Study also pooled pharmacokinetic data from 11 patients from Romsing 2000. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Varvinski 2001.**
Methods	Case report.
Participants	13 year old girl with Soto's syndrome.
Interventions	Orthopaedic surgery, rectal diclofenac (2mg/kg) for acute pain.
Outcomes	Post-operative complications.
Notes	Description of anaesthesia in difficult case. Child received diclofenac and did not have a serious adverse event. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Viitanen 2000.**
Methods	Randomised controlled trial.
Participants	80 children (ASA 1-3) undergoing adenoidectomy with or without myringotomy.
Interventions	1. Sevoflurane 8% (40). 2. Halothane 5% (40). All received rectal diclofenac 12.5mg.
Outcomes	1. Recovery characteristics. 2. Adverse events.
Notes	Evidence of monitoring for adverse events, 80 patients received diclofenac, no serious adverse events. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Vuori 2004.**
Methods	Randomised controlled trial.
Participants	51 children (ASA 1-2) undergoing elective major abdominal, thoracic or orthopaedic surgery.
Interventions	1. Intravenous diclofenac1.5mg/kg followed by rectal diclofenac 2mg/kg twice daily (17). 2. Intravenous oxycodone 0.1mg/kg followed by a continuous infusion of 0.03mg/kg/hr (16). 3. Epidural bupivacaine 0.25% 0.1-0.2mL/kg followed by an epidural infusion of bupivacaine 0.125% plus fentanyl 50μg (15). Each treatment lasted for three days after the operation.
Outcomes	1. Systemic and local immune response. 2. Pain. 3. Diclofenac pharmacokinetics. 4. Adverse events.
Notes	Main study focus on immune response to surgery, no detail on adverse effvents. However, adverse events were monitored for and one serious adverse event (bleeding requiring transfusion) occurred in diclofenac group. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.
Study	**Walmsley 1997.**
Methods	Audit
Participants	30 children undergoing ENT surgery.
Interventions	Soluble oral diclofenac 12.5mg or 25mg.
Outcomes	1. Pain.
Notes	Wrote to author, no serious adverse events occurred. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Watters 1988.**
Methods	Randomised controlled trial, investigator blind.
Participants	75 children (ASA 1-2) undergoing tonsillectomy.
Interventions	1. Intramuscular diclofenac 1mg/kg (25). 2. Pethidine 1mg/kg (25). 3. No analgesia (25).
Outcomes	1. Pain. 2. Drowsiness. 3. Vomiting.
Notes	Pethidine rescue analgesia used. Adverse events monitored for and no serious adverse events occurred. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Adequate.

Table 4.4 (continued): Characteristics of included studies (Appendix 7.10).	
Study	**Wennstrom 2002.**
Methods	Randomised controlled trial.
Participants	50 children (ASA 1-2) undergoing strabismus surgery.
Interventions	1. Rectal diclofenac 1mg/kg (25). 2. Intravenous morphine 0.05mg/kg (25).
Outcomes	1. Pain. 2. Nausea and vomiting.
Notes	Risk of bias as no allocation blinding, morphine used for rescue analgesia. Cannot include in comparative analysis as gives number of episodes not number of patients with vomiting. Adverse events monitored for and no serious adverse events occurred. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Inadequate.
Study	**Williams 2002.**
Methods	Randomised controlled trial.
Participants	98 children (ASA 1-2) undergoing andenotonsillectomy.
Interventions	1. Intramuscular codeine 1.5mg/kg (48). 2. Intramuscular morphine (0.15mg/kg) (48). All patients received rectal diclofenac 1mg/kg.
Outcomes	1. Pain. 2. Adverse events. 3. CYP2D6 genotype.
Notes	Two withdrawals, neither received diclofenac. Adverse events monitored for, 96 received diclofenac and no serious adverse events occurred. Safety quality: moderate.
Allocation concealment	Not applicable.
Study	**Yamomoto 1994.**
Methods	Comparative study.
Participants	74 children undergoing minor surgery.
Interventions	1. Sevoflurane. 2. Isoflurane. 3. Halothane. Rectal diclofenac 1mg/kg was given to 23 patients.
Outcomes	1. Pain. 2. Adverse events.
Notes	Adverse events monitored for and no serious adverse events occurred. Asthmatics: unknown. Safety quality: moderate.
Allocation concealment	Not applicable.
ASA = American Society for Anesthesiologists physical status classification system.	

Allocation concealment was considered adequate if the method of concealment was given, and this method ensured that adverse event assessors were unaware of the treatments given, inadequate if the method did not obviously ensure that adverse event assessors were unaware of treatments, and unclear if blinding was mentioned but no method was given. For studies where diclofenac was not being compared with another treatment, and therefore only the incidence of serious adverse events was of interest, allocation concealment was not applicable.

Reasons for rejecting papers are given in table 4.5.

Table 4.5: Papers rejected from final analysis (Appendix 7.10).	
First author and year	**Reason for rejection**
Bano 2004.	Diclofenac as rescue analgesia, not all patients received it cannot tell numbers.
Baroni 1983.	Adult and paediatric patients, cannot separate out data on children.
Camera 1992.	Adult and paediatric patients, cannot separate out data on children.
Campbell 1990.	Adult patients only.
Castro 1995.	Translated from Spanish – adult patients.
Courtney 2001.	Adult and paediatric patients, cannot separate out data on children.
Fender 1992.	Translated from French – adult patient.
Garcia-Alonso 1990.	Adult patients only.
Keohane 1994.	Not all patients received diclofenac, cannot separate out those who did.
Kurimoto 1993.	Adult and paediatric patients, cannot separate out data on children.
Hernandez-Llenas 1997.	Adult patients.
Hicklin 1999.	'Most' children given diclofenac, wrote to authors, no response.
Karachalios 1992.	Adult patients only.
Kurimoto 1993.	Translated from Japanese - not all patients received diclofenac, cannot separate out those who did.
Kuzelova 2004.	Accidental intoxications, not a study of acute pain in children.
Lau 2002.	Adult patients only.
Lemelle 1998.	Translated from French – review article.
Machida 2004.	Adult and paediatric patients, cannot separate out data on children.
Majid 2004.	Number of patients receiving diclofenac unclear, wrote to authors, no response.
Mannion 1994.	No mention of adverse events, wrote to authors, no response.
Manuksela 1991.	Review article.
Marczyk 1992.	Translated for Portugese - adult patients.
McEwan 2000.	Diclofenac as rescue analgesia, not all patients received it and cannot separate out adverse event data in the ones that did.
Miralles 1987.	Adult patients only.
Mostaque 1998.	No mention of adverse events, wrote to authors, no response.
Nordbladh 1991.	Adult and paediatric patients, cannot separate out data on children.
Ozcan 2002.	Adult patients only.
Pendeville 2001.	Adult and paediatric patients, cannot separate out data on children.
Roelofse 1993.	Adult patients only.
Roelofse 1996.	Adult patients only.
Romej 1996.	Paitients did not receive diclofenac.
Romsing 2001.	Pharmacokinetic data from Romsing 2000 – further analysis on same study/patients.
Rozhkova 1983.	Translated from Russian – adult patients only.
Schaller 1998.	Case reports of NSAID renal toxicity in children – none were diclofenac.
Shah 2001.	Number of patients receiving diclofenac unclear, wrote to authors, no response.
Sheppard 1993.	Not all patients received diclofenac, cannot separate out those who did.
Stanley 2002.	Case report, child received diclofenac but cannot ascertain when or what dose. Adverse events mentioned but no link to diclofenac made in text.
Teiria 1994.	Patients received diclofenac or ibuprofen, cannot tell numbers receiving diclofenac.
Taneja 2004.	Adult and paediatric patients, not all received diclofenac and cannot separate out data on children.
Thomaser 2004.	Translated from German – 70 dropouts, cannot tell total number of children exposed to diclofenac.
Valladares 2004.	Adult and paediatric patients, cannot separate out data on children.
Van den Berg 1999.	Adult and paediatric patients, cannot separate out data on children.
Verheggen 1994.	Adult and paediatric patients, not all received diclofenac and cannot separate out data on children.
Walton 1993.	Adult patients only.

4.5.2 Incidence of Serious Adverse Drug Reactions (Quantitative Analysis)

In total 57 studies (of those listed in table 4.4) were retrieved that provided the number of children exposed to diclofenac (denominator) along with evidence that adverse events were monitored for. These studies contained data on 2760 children, none of whom had a serious adverse event that the authors attributed to diclofenac. A summary of the serious adverse events recorded in studies is given in table 4.6.

Table 4.6: Summary of serious adverse events from quantitative analysis (none of these events were considered to be related to diclofenac by the study authors).

Study	Number of events	Description
Fischer 1992.	1	Unclear as refers to table grouping moderate/severe as rated by patient. Reaction is one of: vomiting, headache, stomach pain or erythema.
Kokinsky 1999.	1	Protracted vomiting requiring prolonged hospitalisation.
Kokinsky 1999.	1	Bleeding after penile surgery requiring re-operation.
McGowan 1998.	2	Late haemorrhage after day-case circumscision requiring hospital admission.
Mendham 1996.	3	Haemorrhage after tonsillectomy requiring re-operation.
Oztekin 2002.	1	Bleeding after tonsillectomy requiring prolonged hospitalisation.
Tawalbeh 2001.	1	Bleeding requiring re-hospitilisation after tonsillectomy.
Tewary 1993.	6	Bleeding causing prolonged or re-hospitalisation after tonsillectomy.
Thiagarajan 1993.	1	Bleeding requiring re-operation after tonsillectomy.
Vuori 2004.	1	Bleeding requiring blood transfusion following major surgery.
Total	18	

4.5.3 Case Reports (Qualitative Analysis)

Four case reports and the details of three serious adverse events that were attributed to diclofenac from a case-note review were identified, details of which were given in table 4.4. These were 'Lai 2005' (tissue necrosis following intramuscular injection), 'Nicolic 2001' (tubulointerstitial nephritis), 'Seaton 2000' (gastrointestinal vasculitis), 'Sen 2004' (fatality from allergic-type reaction) and 'Robinson 1994' (three cases of post-tonsillectomy bleeding attributed to diclofenac in retrospective review). In addition, 77 case reports were provided by the Medicines and Healthcare products Regulatory Agency from the Committee of Safety of Medicine Yellow Card scheme. Of these 77 cases, 29 were relating to children treated with diclofenac for chronic conditions, the indication was

unclear for 12 cases, and 36 cases were in patients being treated for acute pain. Eight of the reactions were classified by the reporter as not serious, 49 were not classified, and 20 were reported to be serious. A summary of these Yellow Card reports combined with the literature case reports is shown in table 4.7.

Table 4.7: Summary of case reports.

	Number of case reports		
Diclofenac indication:	**Chronic condition**	**Unknown**	**Acute pain**
Acute allergic-type reaction	-	1	4
Cardiovascular	-	-	1
Central nervous system	5	2	5
Dermatological	7	4	3
Endocrine	1	-	-
Genitourinary	3	-	1
Gastrointestinal - bleeding	3	3	7
Gastrointestinal - general	3	1	5
Haematological	1	-	5
Hepatic	2	-	-
Injection site reactions	-	-	6
Muscular	-	-	2
Renal	-	1	-
Respiratory	4	1	3

- = no reports.

There were two fatalities, one due to an acute allergic-type reaction causing an inflammatory response and bronchospasm, and one gastric bleed causing peritonitis; both in patients being treated with diclofenac for acute pain.

4.5.4 Comparative Studies

Most studies used rescue analgesia so the groups did not receive exactly the same medications. Table 4.8 gives and intention-to-treat type comparative analysis, whereby adverse events are compared by original treatment allocation, whilst acknowledging that the use of supplementary (rescue) analgesia means that the test drug may not be the direct cause of the adverse event.

Table 4.8: Incidence of adverse events in comparative studies where amount of rescue analgesia may differ between groups.

Author	Blinding	Comparator	Number in diclofenac group	Number in comparator group	Number with adverse event in diclofenac group	Number with adverse event in comparator group
Diclofenac versus any other treatment.						
Nausea and/or vomiting:						
Baer 1992	R	Paracetamol	19	15	0	0
Berry 1992	A	Papaveretum	20	20	6	5
Bone 1998	A	No treatment	20	20	2	1
Littlejohn 1996	A	No treatment	19	20	0	1
McGowan 1998	R	No treatment	20	18	0	2
Nishina 2000	A	No treatment	25	25	2	4
Oztekin 2002	A	No treatment	20	20	8	16
Romsing 2000	A	Paracetamol	24	24	1	16
Samarkandi 2005	A	No treatment	30	30	0	0
Tawalbeh 2000	R	Paracetamol	41	39	1	12
Tay 2002	R	Paracetamol	30	33	0	0
Thiagarajan 1993	A	Papaveretum	91	92	40	45
Watters 1988	A	No treatment	25	25	14	19
Totals:			384	381	74	121
Percentage incidence (95% CI) of nausea and/or vomiting:					19(15-24)	32(26-38)
Bleeding requiring medical or surgical intervention:						
Baer 1992	A	Paracetamol	19	25	0	0
Berry 1992	A	Papaveretum	20	20	0	0
Gadiyar 1995	A	No treatment	19	20	0	0
Littlejohn 1996	A	No treatment	19	20	0	0
McGowan 1998	R	No treatment	20	18	1	0
Tawalbeh 2000	R	Paracetamol	41	39	1	1
Thiagarajan 1993	A	Papaveretum	91	92	1	2
Totals:			229	234	3	3
Percentage incidence (95% CI) of bleeds requiring surgical intervention:					1(0.3-4)	1(0.3-4)
Diclofenac versus other NSAIDs.						
Nausea and/or vomiting:						
Malavasi 1991	R	Nimesulide	29	29	4	1
Mendham 1996	A	Tenoxicam	58	63	13	12
Nishina 2000	A	Flurbiprofen	25	25	11	12
Totals:			112	117	28	25
Percentage incidence (95% CI) of nausea and/or vomiting:					25(16-36)	21(14-32)
Bleeding requiring medical or surgical intervention:						
Mendham 1996	A	Tenoxicam	58	63	3	2
Percentage incidence (95% CI) of bleeds requiring surgical intervention:					5(1-15)	3(0.4-11)

A=Adequate.
R=Risk of bias (blinding unclear or inadequate).
CI=Confidence interval.

Figure 4.2 gives a tree diagram compiled from data in table 4.8 showing the incidence of nausea and vomiting in groups randomised to diclofenac (treatment) or any other non-NSAID (control).

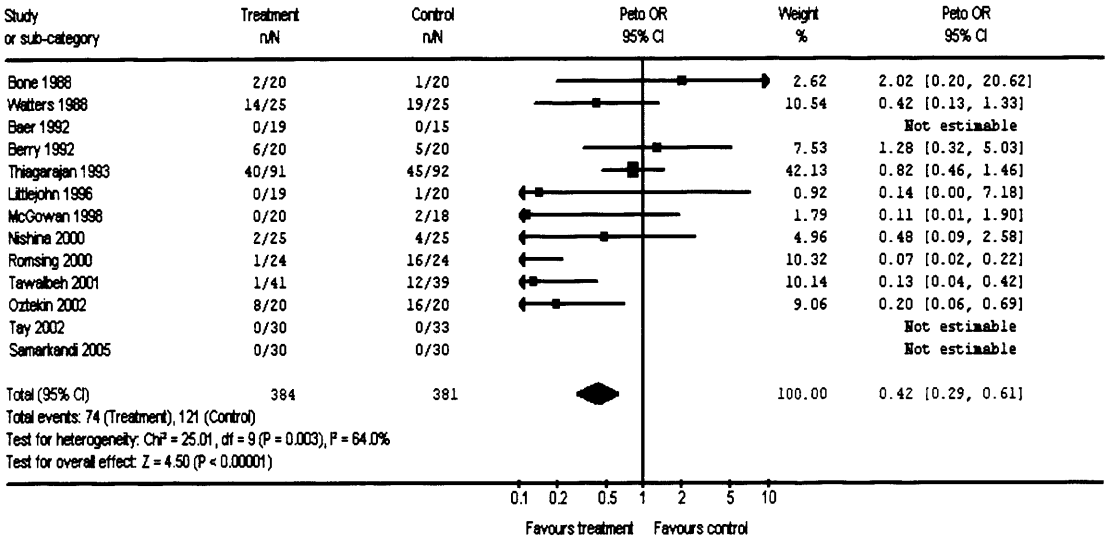


Figure 4.2: Tree-diagram of the incidence of nausea and vomiting in groups randomised to diclofenac (treatment) or any other non-NSAID (control).

Where a paper gave multiple comparisons, diclofenac versus no treatment was chosen. An overview of the included comparisons for nausea and/or vomiting rates where the only difference between groups was whether they received diclofenac or a comparator drug is given is table 4.9.

Table 4.9: Incidence of adverse events in comparative studies where treatment is identical between groups except study medication.

Author	Blinding	Comparator	Number in diclofenac group	Number in comparator group	Number with adverse event in diclofenac group	Number with adverse event in comparator group
Nausea and/or vomiting:						
Romsing 2000	A	Paracetamol	24	24	1	16
*Thiagarajan 1993	A	Papaveretum	91	92	9	3
Totals:			115	116	10	19
Percentage incidence (95% CI) of nausea and/or vomiting:					9(4-16)	16(10-26)
Bleeding requiring medical or surgical intervention:						
Romsing 2000	A	Paracetamol	24	24	0	0
*Thiagarajan 1993	A	Papaveretum	91	92	0	0
Totals			115	116	0	0

*Only the immediate post-operative period data were used as some patients received papaveretum and paracetamol supplementary analgesia after this time. Three children returned to the operating theatre with bleeding, one from the diclofenac group and two from the papaveretum group after rescue analgesia was administered to some patients, see table 4.8.

A=Adequate.
R=Risk of bias (blinding unclear or inadequate).
CI=Confidence interval.

4.6 Discussion

The majority of studies included in this review were on diclofenac being used for post-operative pain in children, despite the fact that any acute-pain setting was searched for. This probably reflects the fact that that conditions such as renal colic and migraine are relatively uncommon in children, and that such sudden onset conditions are difficult to study in a clinical trial. Conversely post-operative pain is common in most children undergoing surgery, and surgery is generally planned and undertaken in a controlled environment that lends itself to clinical studies. Whilst some of the prospective clinical trials only used patients with mild or no systemic disease as denoted by the American Society of Anaesthesiologists (ASA) score (Owens WD, 2001), diclofenac would often be avoided in patients with severe systemic diseases such as renal failure in clinical practice. The data are therefore likely to reflect the population of children who routinely receive diclofenac for acute pain.

In common with the observational study (Chapter Three) none of the serious adverse events (table 4.6) could be readily attributed to diclofenac, and were typical of serious adverse events seen in children following surgery. In total 2760 children were given at least one dose of diclofenac for acute pain. Combining this result with the findings from the observational study (Chapter Three), a total of 3140 patients were prescribed diclofenac without a single serious adverse event such as bronchospasm, gastrointestinal bleeding or acute renal failure occurred that could be recognised as being caused by diclofenac. This means that the incidence of such effects must be less than one in 1047 (less than 0.1 percent) (Hanley JA & Lippman-Hand A, 1983). The incidence of gastrointestinal bleeding in adults receiving short-duration diclofenac treatment has been reported as 0.16 percent (Catalano MA, 1986); an interesting result of this literature review suggests that diclofenac-induced gastrointestinal bleeding is potentially less common in children than adults. Possible reasons for this are that the majority of paediatric trials used diclofenac suppositories, meaning that any local gastrointestinal irritation would be confined to the rectal and distal colon areas which have contents of higher pH than the stomach, possibly making ulceration less likely. A second possibility is that most children received a single dose only, whereas the adult 'short-term' studies included short trials for rheumatoid arthritis (Catalano MA, 1986), suggesting that longer courses of treatment were used, possibly increasing the risk of gastrointestinal bleeding.

The qualitative analysis revealed that similar types of serious diclofenac adverse reactions occur in children and adults. Whilst spontaneous case reports do not give the incidence of adverse effects and are known to greatly under-report recognised drug-induced morbidity and mortality, it has been shown that the distribution of types of reaction in case reports can mirror their incidence in well conducted clinical trials (Loke YK et al, 2004). Not only the types, but also the distribution of case reports were therefore investigated. Yellow Card case reports were provided on diclofenac adverse events in children, regardless of indication and often whether the reaction was considered serious or not. Most of the reactions reported were known adverse effects to diclofenac, probably indicating that children suffer from similar types of adverse effects to adults. The injection site reactions, some of which were serious and required prolonged medical treatment, were all from intra-muscular administrations and are a major reason why this route is now rarely used. Seven

cases of gastrointestinal bleeding were in children treated with diclofenac for acute pain. The fact that relatively more reports on gastrointestinal bleeding were recovered could suggest that of the serious reactions, this is the most common. However, it is possible that as gastrointestinal bleeding is a known adverse reaction caused by NSAIDs, this reaction is more readily attributed to diclofenac meaning relatively more reports are made.

Dermatological reactions were the next most commonly reported, although none of these were classified as serious by the reporter. As mentioned in Chapter Three, there can be multiple causes for minor dermatological reactions, and probably the best way to ascertain if there is a relationship with drug administration would be with a rechallenge. As none of the reported cases mentioned rechallenge, it is unclear as to whether diclofenac was actually causative. In addition to medications, dermatological reactions can be caused by a number of factors including viral infections, external contact with allergens, and foods.

Five central nervous system reactions were reported in children being treated with diclofenac for acute pain, these were dizziness (two cases), abnormal behaviour (two cases) and agitation (one case). Diclofenac is known to have central antinociceptive activity (Burian M & Geisslinger G, 2005) and expression of both COX-1 and COX-2 is constitutive in the central nervous (Samand TA et al, 2001), so it possible that the inhibition of certain prostaglandins may cause such adverse effects. Once again however, without rechallenge to confirm the reoccurrence of such non-specific symptoms, it is difficult to determine with any confidence whether diclofenac is causative. The final interesting finding from the case reports was that only one child suffered a renal adverse event. This may suggest that such events are very rare, or as if diclofenac-induced renal pathology was common, it could be expected that more case reports would have described it (Loke YK et al, 2004).

By including comparative studies this literature review was able to further investigate the incidence of common adverse reactions caused by diclofenac. Nausea and/or vomiting and bleeding were the only specific adverse events that several studies reported on to allow pooled comparison. The quality of adverse event reporting in the included studies was generally moderate with most clearly indicating that adverse events were monitored for,

and dropouts were often accounted for. No study used causality assessment and no placebo control group was used meaning no study was rated as high quality for adverse event reporting (table 4.3). The six studies that compared diclofenac with no treatment were generally single blind with observers unaware of the treatments given in the operating theatre. For the analysis of nausea/vomiting and bleeding, most of the comparative studies that reported adverse events also used rescue analgesia, often with opiates, as a measure of analgesia. In each case it was impossible to determine whether or not the patients with the adverse event had also received rescue analgesia. This meant that the two groups were not treated in an identical fashion, which could cause differing incidence of adverse events not related to the study medication.

Table 4.9 shows there were only two studies, both in patients who had undergone tonsillectomy surgery, comparing diclofenac with another treatment and reporting nausea and vomiting where the only difference between groups was the administration of diclofenac or comparator. There was no significant difference in incidence of nausea and/or vomiting between these two groups, suggesting that diclofenac is unlikely to be more emetogenic than either paracetamol or papaveretum. As most studies used rescue analgesia, and it was not possible to tell whether patients having adverse events did or did not receive rescue analgesia, the comparative analysis in table 4.8 and figure 4.2 is a similar to an intention-to-treat analysis. Differences in adverse event rates are compared between the two groups (diclofenac and non-diclofenac), but the cause of these adverse events may not be directly linked with treatment allocation. For example, it could be that diclofenac provided better analgesia, meaning more patients in the control group received rescue analgesia, which in itself could be emetogenic, influencing the vomiting rate more in one group then the other. Even where rescue analgesia is not used, it has been suggested that adequate analgesia can decrease the incidence of nausea in the peri-operative period (Michaloliakou C et al, 1996), meaning differences in efficacy can possibly influence differences in adverse events.

For patients and parents, the exact cause of a distressing complication such as nausea and/or vomiting is probably irrelevant, and so a comparison of all randomised studies, regardless of the use of rescue analgesia was performed. Figure 4.2 shows that patients

allocated to receive diclofenac had significantly less nausea and/or vomiting than those allocated another non-NSAID treatment. This result does not necessarily indicate that diclofenac is less emetogenic than the comparators, but being allocated to the diclofenac group did decrease nausea and/or vomiting, probably due to a combination of factors including the amount of rescue analgesia required, degree of analgesia and the emetogenic properties of treatments administered. The comparison of nausea and/or vomiting rates between NSAIDs was similar (table 4.8) and the overall incidence of nausea and/or vomiting in patients receiving diclofenac as part of an analgesic regimen for peri-operative pain was found to be between 16 and 26 percent, similar to the incidence (95 percent confidence interval) seen in the observational study (Chapter Three) of 15(12-20) percent. Combining all of the patients who received diclofenac from the literature review regardless of the comparator (table 4.8) with the observational study gives 827 patients (Malavasi paper excluded because it was not a study on peri-operative pain), of whom 154 suffered from nausea and/or vomiting. The incidence (95 percent confidence interval) of nausea and/or vomiting in children receiving diclofenac analgesia for peri-operative pain is therefore 19(16-22) percent.

Most of the studies that looked for bleeding gave subjective measures of blood loss, whilst some did try to quantify the amount of blood loss through suction bottle volume or the difference between the weight of swabs before and after surgery. The significance of a subjective measure of blood loss is unclear and the accuracy of measured blood loss was reflected in the large ranges quoted and the fact that swallowed blood (in tonsillectomies) and blood absorbed by surgical drapes could not be accounted for. It was therefore decided to include only bleeding which required intervention such as reoperation. Table 4.9 shows there was no difference in the incidence of bleeding requiring intervention in patients receiving diclofenac. This confirms the finding of previous reviews on bleeding in paediatric tonsillectomy with NSAIDs: at standard doses NSAIDs do not appear to increase the propensity for bleeding (Cardwell M et al, 2005, Romsing J & Walther-Larsen S, 1997).

The final question raised during the observational study (Chapter Three) was: what is the incidence of bronchospasm in asthmatic children treated with diclofenac for acute pain?

Most studies did not mention asthma, and of the ones that did, a large proportion excluded asthmatic children. No study gave a number of asthmatic children exposed to diclofenac, or made reference to bronchospasm induced by diclofenac in an asthmatic child. Where authors were contacted for other reasons, all were asked about the number of asthmatic children included in their studies and none could give an actual figure. Interestingly, some responders who were unable to give the numbers of asthmatics given diclofenac in their studies, gave their opinion on diclofenac or NSAIDs and asthma. The following is a list of direct quotes from all authors who volunteered their opinion on the subject:

"Usually we are careful not to give NSAIDs to children with asthma."
Dr Kokinsky (Anaesthetist), corresponding author of 'Kokinsky 1999'.

"There were several children [included in the study] with mild asthma and there were no respiratory problems in these children. I am unable to give numbers at this time. Only one child was excluded from the study because of severe asthma which had required several hospital admissions."
Dr Mendham (Anaesthetist), corresponding author of 'Mendham 1999'.

"I do three to four paediatric lists per week. In 10 plus years as a consultant I have given every child NSAIDs (ibuprofen or diclofenac) even if they are asthmatic. I have avoided the very very few who say they are allergic. I have witnessed only one child (13 year old girl) to get bronchospasm. She also had a general anaesthetic and morphine so I cannot be sure it was the NSAID. I think NSAIDs are safe in asthmatic children."
Dr Carr (Anaesthetist), corresponding author of 'Mukherjee 2001'.

"I had quite forgotten about this work so it was a pleasure to be reminded about it. It was not published as a full paper (hence the letter). After some rummaging in my cupboards I do still have the data. Such data however does not record if the children were asthmatic or not. No doubt some of them would have been as about 15 percent of our patients are asthmatic. We do not treat them any differently and give diclofenac to them all. No patients in the study group who received diclofenac

had any serious adverse event. In 12 years of paediatric ENT [ear nose and throat] with over 4000 patients receiving diclofenac, many of whom are asthmatic, I have experienced no problems."
Dr Semple (Anaesthetist), corresponding author of 'Swanepoel 1999'.

"Exclusion criteria was severe asthma i.e. oral steroids at least twice or admission to hospital for asthma. I still use oral diclofenac ten years on and only exclude moderate to severe asthmatics. No problem as far as I am aware, but very good analgesia."
Dr Walmsley (Anaesthetist), corresponding author of 'Walmsley 1997'.

These quotes were not collected as part of a structured survey on the opinion of anaesthetists as to whether diclofenac is likely to cause bronchospasm in asthmatic children, but they probably do reflect the spectrum of opinion on the matter. Unfortunately this literature review could not identify the prevalence of diclofenac-induced bronchospasm in asthmatic children as these children were either excluded, or the data (number of asthmatic children included) not reported or seemingly collected in most studies. The experience of Drs Carr and Semple would suggest that this reaction is infrequent; a study to investigate how infrequent diclofenac-induced bronchospasm in asthmatic children certainly seems to be warranted.

4.7 Conclusions

The main finding from the quantitative analysis in this review was that the incidence of serious adverse reactions caused by diclofenac was less than 0.1 percent. This means that children seem no more likely than adults to experience a serious adverse drug reaction to diclofenac. The qualitative review did however find that children do suffer similar types of serious adverse reactions, such as gastrointestinal bleeds and allergic-type reactions, and unfortunately two fatalities caused by diclofenac have been seen in children. With some estimates suggesting that less than ten percent of serious adverse reactions are reported, it seems likely that there are several more children who have died as a result of being given diclofenac for acute pain. This must be balanced against the analgesic benefits gained by many thousands of children who suffer no adverse effects at all.

With the vast majority of comparative studies being on peri-operative pain, it was not possible to pool comparisons where the only treatment was either diclofenac or placebo/a comparator. It was therefore not possible to calculate the incidence of nausea and/or vomiting directly caused by diclofenac as a proportion of the study population (children having an operation under general anaesthetic) are likely to routinely suffer from this effect. Using diclofenac as part of the analgesic regime decreased the incidence of nausea and/or vomiting, probably due to it providing good analgesia and sparing the need for rescue analgesics. The finding that diclofenac does not increase the incidence of clinically significant bleeding confirms previous studies (Cardwell M et al, 2005).

To conclude, the observational safety study and systematic review have shown that children suffer similar types of adverse reactions to adults, that serious adverse reactions are rare (as with adults), that no increase in bleeding has been proven when diclofenac is used in the peri-operative period, and that diclofenac used as part of the analgesic regime in the peri-operative period reduces the incidence of nausea and/or vomiting.

Chapter FIVE: Ontogeny of CYP2C9

5.1 Introduction

Whilst previous studies in this thesis have sought to answer clinical questions on diclofenac safety and dosing, it is also important that a contribution to knowledge on paediatric drug handling is made. Increased understanding of paediatric drug handling will ultimately lead to better predictions on the pharmacokinetics and pharmacodynamics of new drugs in children, meaning that future clinical trials can be designed to maximise the amount of information gathered from as few patients as feasible. The use of such knowledge may include the building of physiologically-based models to predict drug handling in children (Edgington AN et al, 2006, Johnson TN et al, 2006) prior to designing confirmatory clinical studies, or the investigation of allometric scaling factors, which are currently showing promise in predicting pharmacokinetics and pharmacodynamics across species (Yassen A et al, 2007, Zuideveld KP et al, 2007), and could help predict paediatric pharmacokinetics and pharmacodynamics from adult data (Meibohm B et al, 2005). In addition to predicting the dosing and effects of new drugs, increased understanding of paediatric drug handling will lead to better understanding of dose-response relationships for existing treatments.

One factor important in predicting drug handling in children, especially in the development of physiologically-based pharmacokinetic models, is the expression of drug metabolising enzymes. As mentioned in Chapter One, some drug metabolising enzymes show developmental differences in expression, and this is usually ascertained from liver and/or gut pathology samples (Hines RN & McCarver DG, 2002, McCarver DG & Hines RN, 2002). The next step from such *in vitro* studies is the confirmation of their findings with *in vivo* assessment. Investigating the age at which drug metabolising capacity reaches adult-equivalent levels, using suitable probe drugs, provides a way of confirming or refuting the findings of *in vitro* studies.

CYP2C9 constitutes 18 percent of adult liver phase I enzymes and is responsible for metabolising drugs such as phenytoin, warfarin and tolbutamide (Lee CR et al, 2002). The hydroxylation of diclofenac to 4'-hydroxydiclofenac is also catalysed by CYP2C9 (Tang W, 2003), and so the clearance of diclofenac to 4'-hydroxydiclofenac could prove a useful

marker of CYP2C9 expression. To date, a single *in vitro* study investigating the developmental expression of CYP2C9 using hepatic microsomal specimens has been undertaken (Koukouritaki SB et al, 2004). Samples from 237 subjects aged eight weeks gestation to 18 years were analysed for CYP2C9 content by Western blotting using CYP2C9 antibodies. A further assessment of CYP2C9 activity was also undertaken using diclofenac conversion to 4'-hydroxydiclofenac. Both analyses in this study found that CYP2C9 expression gradually increased during gestation, with a rapid increase immediately after birth. During the neonatal period and early infancy CYP2C9 expression seemingly continued to increase and was probably adult-equivalent by five months of age (Koukouritaki SB et al, 2004). Given that this study used frozen liver samples, and that CYP2C9 is known to be expressed in extrahepatic regions such as the small intestine (Ding X & Kaminksy LS, 2002), the question raised is: how well do these findings reflect CYP2C9 expression *in vivo*?

In the pharmacokinetic study (Chapter Two), each paediatric serum sample was assayed for both diclofenac and 4'-hydroxydiclofenac. Using this data, a compartmental pharmacokinetic modelling analysis with NONMEM will be undertaken to ascertain 4'-hydroxydiclofenac appearance, which will be used as an indicator of *in vivo* CYP2C9 expression. This measure of CYP2C9 expression will then be compared between children of different ages. The *in vitro* data suggested that CYP2C9 expression should be adult-equivalent after five months of age (Koukouritaki SB et al, 2004). The patients from the pharmacokinetic study (Chapter Two) were aged one to 12 years, so theoretically no differences should be found in diclofenac 4'-hydroxylation with age in these children, as CYP2C9 should already be adult-equivalent.

Using pharmacokinetic modelling of oral diclofenac clearance to 4'-hydroxydiclofenac as a probe for CYP2C9 expression presents an interesting challenge. This is because some 4'-hydroxydiclofenac will be formed during first-pass metabolism, and some will be formed from the free diclofenac fraction circulating in the blood. A simple representation of this process is given is figure 5.1:

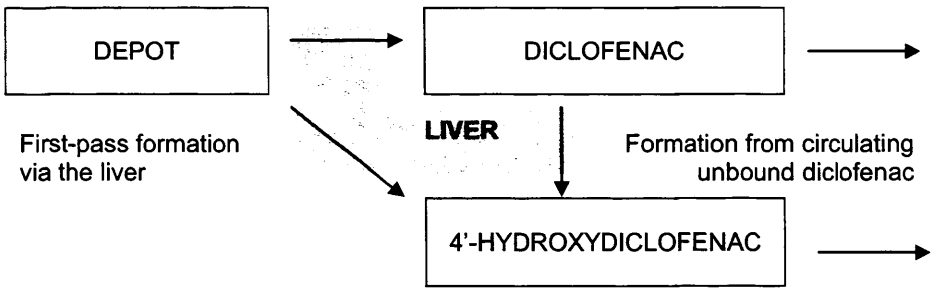


Figure 5.1: Schematic diagram describing routes of 4'-hydroxydiclofenac formation.

This physiologically plausible model of 4'-hydroxydiclofenac formation cannot be used in the compartmental analysis of serum concentrations because it contains unidentifiable parameters (Venot A et al, 1987). From serum concentrations, it is not possible to tell how much 4'-hydroxydiclofenac is being produced from the first-pass effect, or how much from unbound circulating diclofenac, meaning the rate of formation from the two routes cannot be separately estimated. The challenge therefore is to develop an empirical model that adequately explains the observations, and use this to investigate how 4'-hydroxydiclofenac appearance in the circulation changes with age.

The empirical structural model will assume that diclofenac clearance to 4'-hydroxydiclofenac occurs from the central compartment, a schematic overview is given in figure 5.2. This method has the advantage that the formation clearance (appearance in the circulation) of 4'-hydroxydiclofenac is an identifiable (if not entirely physiologically plausible) parameter from serum concentrations. In addition, the formation of 4'-hydroxydiclofenac will be affected by the variability in diclofenac absorption, which is due to pH-dependent dissolution variability in the gastrointestinal tract. This variability will be accounted for by using the same absorption model as in the pharmacokinetic study (Chapter Two).

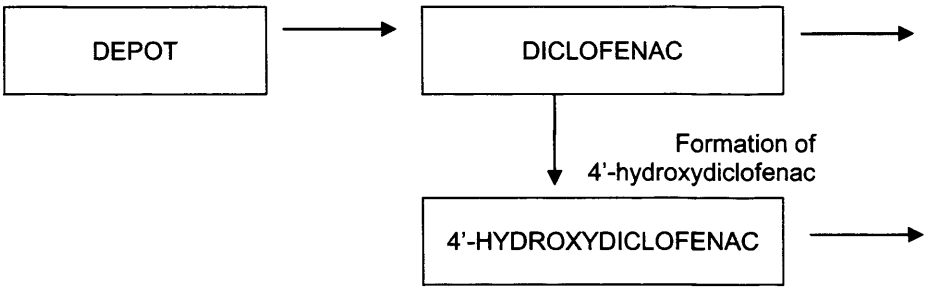


Figure 5.2: Schematic pharmacokinetic model to be used to describe 4'-hydroxydiclofenac appearance in the circulation.

The rate of formation of 4'-hydroxydiclofenac at the site of metabolism follows saturable Michaelis-Menten kinetics (Koukouritaki SB et al, 2004). This rate of formation can be described by the Michaelis-Menten equation (5.1) (Rowland M & Tozer TN, 1994):

$$\frac{\delta C_D}{\delta t} = \frac{Vm_{CYP2C9} \times C_D}{Km_{CYP2C9} + C_D} \qquad \text{Equation 5.1}$$

Where:

Vm_{CYP2C9} = Maximum rate of substrate metabolism.
C_D = Free diclofenac concentration at the enzyme active site.
Km_{CYP2C9} = Michaelis-Menten constant (free diclofenac concentration at $Vm_{CYP2C9}/2$).

In this expression, Vm_{CYP2C9} is directly proportional to CYP2C9 concentration, and Km_{CYP2C9} is inversely related to enzyme affinity (Rowland M & Tozer TN, 1994). As no data on diclofenac concentrations at the point of CYP2C9 metabolism, be it in the gastrointestinal tract or liver, are available, it is unlikely that realistic estimates of Vm_{CYP2C9} and Km_{CYP2C9} will be obtainable. However, it is possible that the appearance of 4'-hydroxydiclofenac in the blood, as measured by serum concentrations, may follow a saturable Michaelis-Menten like process whereby CYP2C9 capacity is reached and the rate of entry into the circulation becomes zero-order. For this reason, the first model for the rate of entry of 4'-hydroxydiclofenac into the circulation will follow a Michealis-Menten rate as shown in equation 5.2:

$$\frac{\delta C_{Dserum}}{\delta t} = \frac{Vm_{app} \times C_{Dserum}}{Km_{app} + C_{Dserum}} \qquad \text{Equation 5.2}$$

Where:

Vm_{app} = Maximum rate of 4'hydroxydiclofenac appearance (nmol/hr).
C_{Dserum} = Diclofenac serum concentration (nmol/L).
Km_{app} = Michaelis-Menten constant (diclofenac serum concentration at $Vm_{app}/2$) (nmol/L).

The appearance of 4'-hydroxydiclofenac in the serum does not only depend on CYP2C9 activity, but also on blood flow transporting the substrate to the site of metabolism, and the product back to the venous circulation. The fact that CYP2C9-mediated diclofenac 4'-hydroxylation has a high turnover rate (Rettie AE & Jones JP, 2005), and that the overall hepatic extraction ratio of diclofenac in humans is possibly as low as 40 percent (Davies NM & Anderson KE, 1997), suggests that 4'-hydroxydiclofenac appearance in the circulation may be limited by hepatic perfusion rate and therefore follow a first-order process (Rowland M & Tozer TN, 1994). This is because the conversion to 4'-hydroxydiclofenac is such an efficient process, that the rate of entry into the circulation would be limited by blood flow to and from the site of metabolism, rather than enzyme activity. For this reason, both first-order and Michaelis-Menten models will be investigated.

The rate of 4'-hydroxydiclofenac appearance by a first-order process will be defined by equation 5.3:

$$\frac{\delta C_{Dserum}}{\delta t} = C_{Dserum} \times K_{4OHapp} \qquad \text{Equation 5.3}$$

Where:

K_{4OHapp} = Rate constant of 4'-hydroxydiclofenac appearance (hr^{-1}).

In this case K_{4OHapp} is an elimination rate constant describing the rate of diclofenac transformation to 4'-hydroxydiclofenac.

Whether the Michealis-Menten or first-order model is chosen will depend on which one best describes both diclofenac and 4'-hydroxydiclofenac observations, and will be evaluated in a similar manner to that used in the diclofenac pharmacokinetic study (Chapter Two). The aim of model building will be to predict the AUC for both diclofenac and 4'-hydroxydiclofenac in each paediatric patient. The predicted AUC ratio between diclofenac and 4'-hydroxdiclofenac will be used as a measure of CYP2C9 activity, with higher ratios indicating proportionally less 4'-hydroxydiclofenac being formed, and therefore lower CYP2C9 expression. Comparison of diclofenac and 4'-hydroxydiclofenac AUC ratio has been used in several studies investigating the influence of CYP2C9 genotype on the formation of 4'-hydroxydiclofenac (Morin S et al, 2001, Shimamoto J et al, 2000, Yasar U et al, 2001).

A potential confounding factor in investigating the developmental expression of CYP2C9 is genotype. CYP2C9 is one of four functional enzymes in the CYP2C subfamily and is encoded by a polymorphic gene of 55kb on chromosome 10. Single nucleotide polymorphisms (SNPs), especially in exon 7 which codes for amino acids in the substrate recognition portion, can produce variants with reduced enzyme activity (Lee CR et al, 2002). To-date, 30 alleles of the CYP2C9 gene have been identified (www.cypalleles.ki.se/cyp2c9.htm), and a summary of the properties of the first 12 are given in table 5.1.

The reason that genotype could potentially confound this study on developmental expression of CYP2C9 is that if more patients in one age group have a 'poor metaboliser' genotype, which translates into reduced CYP2C9 activity *in vivo*, this may lead to the false conclusion that CYP2C9 expression changes with age. For this reason, the patients will all be genotyped for CYP2C9. In previous adult studies seeking a correlation between CYP2C9 genotype and diclofenac 4'-hydroxylation, the only variants looked for were *2 and *3. As can be seen in table 5.1, the *5 and *11 genotypes also have potentially decreased activity, and this study will therefore also look for these alleles.

Table 5.1: Major polymorphisms in CYP2C9 and effect on metabolic activity.

Allele	Amino acid change	Allele frequency	*In vitro* activity relative to wild-type	Comments
*1	Wild-type	>74%	Normal	
*2	144 Arg → Cys	11%	No significant reduction in Km, small reduction in Vm.	Reductions in activity may be substrate specific.
*3	359 Ile → Leu	6%	3-fold Km decrease in diclofneac 4'-hydroxylation.	All substrates show decreased activity.
*4	359 Ile → Thr	1 patient	Decreased activity?	Very rare.
*5	360 Asp → Glu	3%	5-fold Km decrease in diclofneac 4'-hydroxylation.	Not seen in caucasian patients?
*6	DELETION	0.6%	None – frameshift produces inactive protein	
*7	19 Leu → Ile	?	No significant decrease in catalytic activity with tolbutamide methylhydroxylation.	
*8	150 Arg → His	?	Slightly higher catalytic activity with tolbutamide methylhydroxylation.	
*9	251 His → Arg	0.5%	No significant decrease in catalytic activity with tolbutamide methylhydroxylation.	
*10	272 Glu → Gly	?	No significant decrease in catalytic activity with tolbutamide methylhydroxylation.	
*11	353 Arg → Trp	1%	3-fold Km decrease in tolbutamide methylhydroxylation.	
*12	489 Pro → Ser	0.5%	0.4-fold Km decrease in tolbutamide methylhydroxylation.	

	= Amino acids in substrate recognition site of protein coded in exon 7
Vm	= Substrate concentration at which all enzyme active sites are occupied.
Km	= Michaelis-Menten constant – concentration to reach 50% of Vm.

Data obtained from:
Dickman LJ et al, 2001, Imai J et al, 2000, Lee CR et al, 2002, Rettie AE & Jones JP, 2005, Suarez-Kurtz G, 2005, Takanashi K et al, 2000, www.cypalleles.ki.se/cyp2c9.htm

5.2 Aims

To explore pharmacokinetic compartmental modelling as a method for assessing CYP2C9 *in vivo* activity using diclofenac as a probe.

To investigate the influence of age on CYP2C9 *in vivo* activity using the metabolism of diclofenac to 4'-hydroxydiclofenac as a surrogate marker.

To investigate the influence of CYP2C9 genotype on *in vivo* metabolic activity using the metabolism of diclofenac to 4'-hydroxydiclofenac as a surrogate marker.

5.3 Objectives

To develop a parsimonious pharmacokinetic model that describes the observed diclofenac and 4'-hydroxydiclofenac concentrations.

To compare 4'-hydroxydiclofenac appearance in the circulation in children of different ages.

To compare 4'-hydroxydiclofenac appearance in the circulation in children with different CYP2C9 genotypes.

5.4 Methods

5.4.1 Diclofenac Administration and Blood Sampling

This study used data collected during the diclofenac pharmacokinetic study (Chapter Two). Oral diclofenac 1mg/kg (Rosemont Pharmaceuticals Ltd., UK) was given to children prior to minor surgery, and up to three blood samples drawn, the time of which was accurately recorded. Serum was extracted and in addition to diclofenac, each sample was also assayed for 4'-hydroxydiclofenac concentration. One extra whole blood sample was collected along with the first sampling point for CYP2C9 genotyping.

The adult volunteer diclofenac data from the pharmacokinetic study (Chapter Two) was also included to allow the dual transit absorption model to be implemented. Each adult volunteer received a single dose of oral diclofenac 50mg (Rosemont Pharmaceuticals Ltd., UK) with blood samples for diclofenac assay drawn at: 0.25, 0.50, 0.75, 1.0, 1.33, 1.67, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 9.0 and 12.0 hours post-dose. Where actual sampling times deviated from this schedule by more than one minute, actual time was recorded.

5.4.2 Diclofenac and 4'-Hydroxydiclofenac Assay

The analytical unit at St George's Hospital, London, undertook assays of the paediatric serum samples. The method used was sequential high performance liquid chromatography followed by mass spectrometer detection (HPLC/MS). Solid phase extraction was used to clean the samples prior to HPLC using an Altima C18 column with methanol and ammonium acetate (5mmol/L) 50:50 (Rathburn Chemicals Ltd., UK) used as the mobile phase. Ketoprofen (Sigma-Aldrich Ltd., UK) was used as internal standard and detection was performed by an AP1400 mass spectrometer with nitrogen as the collision gas. Diclofenac sodium (Sigma-Aldrich Ltd., UK) and 4'-hydroxydiclofenac (Novartis Ltd., Switzerland) were used for calibration. Output was analysed using Analyst (version 1.3.2) software that performed integration of diclofenac and 4'-hydroxydiclofenac detection peaks. The lower limit of detection was 10.1ng/mL and 4.9ng/mL for diclofenac and 4'-hydroxydiclofenac respectively. The intra-assay precision, defined by the percentage coefficient of variation, ranged from 0.81 to 11.78 percent and 1.03 to 16.73 percent for diclofenac and 4'-hydroxydiclofenac respectively, the mean percentage accuracy ranged from 94.16 to 112.59 percent and 94.67 to 109.82 percent for diclofenac and 4'-hydroxydiclofenac respectively.

5.4.3 CYP2C9 Genotyping

CYP2C9 genotyping was undertaken by the Department of Forensic Medicine, University of Helsinki, and used a similar approach to their previous work on CYP2D6 genotyping (Sistonen J et al, 2005). Firstly DNA was extracted from leukocytes in the 1mL whole blood samples using a proprietary kit (EZNA Blood DNA Kit, Omega Bio-Tek). Primers (CYP2C9-F1, CYP2C9R1, CYP2C9-F2 and CYP2C9-R2, see table 5.2) were used to identify and amplify two fragments (covering exons 2 and 3, and exon 7) by polymerase chain reaction (PCR). PCR was conducted using 25μL of reaction mixture containing subject DNA, primers, DNA polymerase and buffer: denaturation at 95°C for seven minutes was followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for three minutes, and a final extension at 72°C for five minutes. PCR products were analysed using 1.5 percent agarose gels to ensure the correct fragments were amplified. The PCR product was then purified to remove primers and extraneous reagents using of 1.0U of Exonuclease I (USB) and 0.1U of Shrimp Alkaline Phosphatase (USB) per 5μL of

PCR product, with incubation at 37°C for 15 minutes and at 80°C for 15 minutes to deactivate the enzymes.

SNaPShot™ (Applied Biosystems) was used to identify alleles of interest. The SNaPShot™ method used detection primers of complementary sequence to that immediately preceding the base of interest (see table 5.2), namely 430C>T (**2*), 1075A>C (**3*), 1080C>G (**5*) and 1003C>T (**11*). Once these detection primers were bound, a single base extension reaction (i.e. extending the primer to the following base which possibly contained the SNP) was undertaken with DNA polymerase and fluorescent nucleotides in the SNaPShot™ ready reaction mixture (Applied Biosystems). Purification to remove unincorporated nucleotides by the addition of 1.0U of calf intestinal phosphatase (Finnzymes) and incubation of the samples at 37°C for one hour and at 75°C for 15 minutes to successively deactivate the enzyme, was followed by detection by capillary electrophoresis (ABI PRISM 3100 genetic analyser); output was analysed using GeneMapper ID (Version 3.1, Applied Biosystems).

Table 5.2: Primers used in the CYP2C9 genotyping process.

Fragment detection primers:	
CYP2C9-F1	5'-TTCGTTTGCTGTTATCTCTGTCTA-3'
CYP2C9-R1	5'-CAACCAGGACTCATAATGAAAGATA-3'
CYP2C9-F2	5'-ACCATCCTCTCTTTAAGTTTGCAT-3'
CYP2C9-R2	5'-ATACTATGAATTTGGGGACTTCG-3'
SNP detection primers:	
430C>T (**2*)	5'-$(T)_4$GGAAGAGGAGCATTGAGGAC-3'
1075A>C (**3*)	5'-$(T)_9$TGCACGAGGTCCAGAGATAC-3'
1080C>G (**5*)	5'-$(T)_{16}$CAGGCTGGTGGGGAGAAG-3'
1003C>T (**11*)	5'-$(T)_{18}$GAACGTGTGATTGGCAGAAAC-3'

The mean and standard-deviation of the age of patients with each genotype were calculated to investigate any potential confounding relationships between genotype and age.

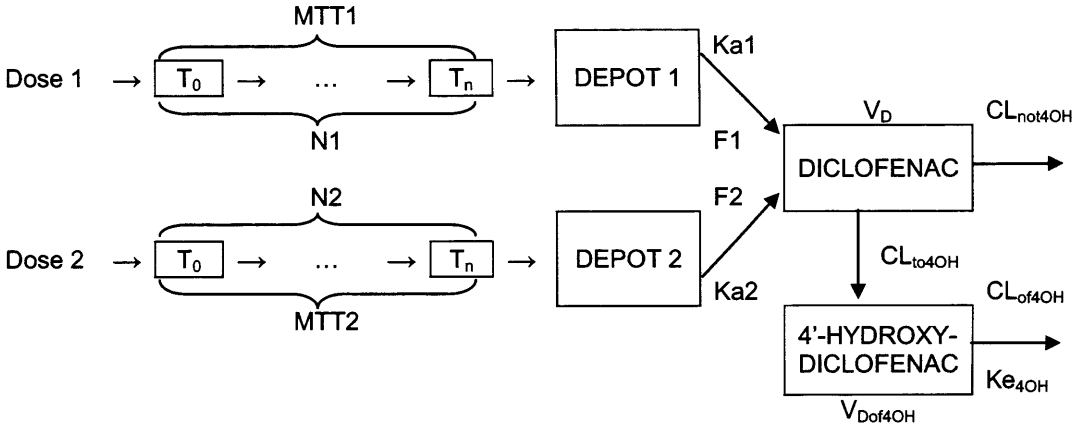
5.4.4 Pharmacokinetic Model Building

Raw plots of diclofenac and 4'-hydroxydiclofenac concentrations were generated in Excel, and rough estimation of 4'-hydroxydiclofenac AUC and slopes of the appearance and elimination curves were used to derive initial parameter estimates. For pharmacokinetic terms relating to diclofenac concentrations, the same initial estimates were used as the final model in Chapter Two. Assay reports of diclofenac and 4'hydroxydiclofenac concentrations were given in ng/mL and diclofenac was dosed in mg of diclofenac sodium. For this reason, all mass units were changed to nanomoles (assuming a molecular weight of 318.13g for diclofenac sodium, 296.15g for diclofenac and 312.15g for 4'-hydroxydiclofenac) and all volume quantities were transformed to litres. The 4'-hydroxydiclofenac concentrations from the paediatric patients were added to the NONMEM-format data file used in the diclofenac pharmacokinetic study (Chapter Two).

The structural pharmacokinetic model was an extension of that used in Chapter Two. An extra compartment to describe 4'-hydroxydiclofenac concentrations was added, with clearance of diclofenac to 4'-hydroxydiclofenac being from the central compartment as shown in figure 5.3. As only concentration measures were available in this study, the amount of diclofenac metabolised to 4'-hydroxydiclofenac, that is to say the 'dose' of 4'-hydroxydiclofenac added to the system, was unknown. For this reason, 4'-hydroxydiclofenac volume of distribution was fixed to a constant value. In adults, after oral administration of a buffered aqueous solution of diclofenac, 4'-hydroxydiclofenac clearance was approximately 10L/hr, and the elimination half-life approximately two hours (Degen PH et al, 1988). The volume of distribution of 4'-hydroxydiclofenac in adults is therefore 30L (V_D=CL/ke=CL/(ln2/$t_{1/2}$)). For this study, a fixed 4'-hydroxydiclofenac volume of distribution of 30L/70kg was used, and this was assumed to vary in a linear way with body weight (Meibohm B et al, 2005).

Pharmacokinetic model building was undertaken using a Dell D600 Notebook with Intel Pentium processor (2.00GHz) running NONMEM (version 6.0) compiled with a Compaq Visual Fortran (version 6.1) compiler. The estimation method chosen in NONMEM was first-order conditional estimation with interaction. Allometric scaling was added to all

clearance and volume fixed effects *a priori* and standardised to a body weight of 70kg (Meibohm B et al, 2005).



Tn = Transit compartment.

The following fixed effects were estimated in NONMEM:

MTT1	= Mean transit time into first depot compartment (hr).
N1	= Number of transit compartments prior to first depot compartment.
F1	= Fraction absorbed from first depot compartment.
$t_{1/2A1}$	= Absorption half-life from first depot compartment (hr) = ln2/Ka1.
MTT2	= Mean transit time into second depot compartment (hr).
N2	= Number of transit compartments prior to second depot compartment.
F2	= Fraction absorbed from second depot compartment (fixed to = 1 – F1).
$t_{1/2A2}$	= Absorption half-life from second depot compartment (hr) = ln2/Ka2.
V_D	= Volume of distribution of diclofenac (L).
CL	= Clearance of diclofenac (L/hr) = CL_{not4OH} + CL_{to4OH}.
CL_{NOT4OH}	= Clearance of diclofenac not including 4'-hydroxylation (L/hr).
CL_{to4OH}	= Clearance of diclofenac to 4'-hydroxydiclofenac (L/hr).
V_{Dof4OH}	= Volume of distribution of 4'-hydroxydiclofenac (fixed to 30L/70kg).
CL_{of4OH}	= Clearance of 4'-hydroxydiclofenac (L/hr) = V_{Dof4OH} x Ke_{4OH}.

Figure 5.3: Schematic diagram of structural model and overview of fixed-effects estimated in NONMEM.

The two models tested for the appearance of 4'-hydroxydiclofenac into the circulation were first-order and Michaelis-Menten. The rate of appearance of 4'-hydroxydiclofenac was modelled using equation 5.3 for the first-order model, and equation 5.2 for the Michealis-Menten model. In the first order 4'-hydroxydiclofenac appearance model, CL_{to4OH} was defined as shown in equation 5.4, in the Michaelis-Menten model CL_{to4OH} was defined as the intrinsic clearance as shown in equation 5.5:

$$CL_{to4OH} = V_D \times K_{4OHapp} \quad \text{Equation 5.4}$$

Where:

CL_{to4OH} = Clearance to 4'-hydroxydiclofenac (L/hr).
V_D = Diclofenac volume of distribution (L).
K_{4OHapp} = Rate constant of 4'-hydroxydiclofenac appearance (hr^{-1}).

$$CL_{to4OH} = \frac{Vm_{app}}{Km_{app}} \quad \text{Equation 5.5}$$

Where:

Vm_{app} = Maximum rate of 4'-hydroxydiclofenac appearance (nmol/hr).
Km_{app} = Substrate (diclofenac) concentration at half the maximum rate of 4'-hydroxydiclofenac appearance (nmol/L).

Inter-individual variability (η) was assumed to be log-normally distributed (so negative parameter values could not be estimated), and a block covariance matrix was used to force clearance and volume terms to co-vary. As diclofenac assays were performed in different laboratories for the adult and paediatric data, the adult samples were plasma whereas the paediatric samples were serum, and the assay variability for diclofenac and 4'-hydroxydiclofenac was different in the paediatric dataset, estimation of residual variability was undertaken separately for adult diclofenac, paediatric diclofenac and paediatric 4'-hydroxydiclofenac concentrations respectively. As in Chapter Two, a proportional residual error model was used for diclofenac concentrations in both adult and paediatric subjects, and models tested for the 4'-hydroxydiclofenac concentrations were proportional, additive and mixed proportional-additive error. Full NONMEM control files for the Michaelis-Menten and first-order appearance models are given in appexdices 7.11 and 7.12.

5.4.5 Pharmacokinetic Model Evaluation

NONMEM output table files were used to generate diagnostic plots in Excel for general structural model and residual error model evaluation. A visual predictive check was conducted, which entailed simulating new data based on distributions derived from the final parameter estimates. A new data structure was constructed, with 100 patients with identical demographic details to the original dataset but sampling times at 0.5, 1, 2, 4, and 8 hours so that a median value at each time point could be derived. Using NONMEM, 100 new datasets based on this data structure were simulated, and the median, fifth and 95th

percentiles plotted on top of the original data. This plot gives a visual measure of model performance and ideally around five percent of the original data should fall above and below the fifth and 95th percentiles of the model-simulated data.

5.4.6 Investigating the Relationship Between Age and Diclofenac:4'-Hydroxydiclofenac AUC Ratio

Once the final pharmacokinetic model was reached, a time point of 12 hours was added to each subject in the data file. It was decided to use 12 hours because the terminal elimination half-life of diclofenac is between one and two hours (Davies NM & Anderson KE, 1997) and the terminal elimination half-life of 4'-hydroxydiclofenac is approximately two hours (Degen PH et al, 1988). By 12 hours, it would therefore be expected that diclofenac and 4'-hydroxydiclofenac were almost completely eliminated. Using the final model, the empirical Bayesian estimated concentration up to 12 hours was predicted for each patient, and concentration-time curve was integrated using NONMEM giving the $AUC_{(0\text{-}12h)}$. The $AUC_{(0\text{-}12h)}$ was estimated for diclofenac and 4'-hydroxydiclofenac, and the ratio of diclofenac $AUC_{(0\text{-}12h)}$:4'-hydroxydiclofenac $AUC_{(0\text{-}12h)}$ calculated for each patient and plotted against age.

5.4.7 Investigating the Relationship Between CYP2C9 Genotype and Diclofenac:4'-Hydroxydiclofenac AUC Ratio

The median ratio of diclofenac $AUC_{(0\text{-}12h)}$:4'-hydroxydiclofenac $AUC_{(0\text{-}12h)}$ was calculated for each genotype group, and a Mann-Whitney test (calculated in SPSS) was used to determine if there was a difference between each genotype and the wild type (*1/*1).

5.5 Results

5.5.1 Raw Concentration Data

Model building used data collected in the pharmacokinetic study that comprised of 558 (206 paediatric, 352 adult) diclofenac concentrations and 206 (all from paediatric patients) 4'-hydroxydiclofenac concentrations (raw data is provided on a CD located in the back cover of this thesis). Figure 5.4 gives the relationship between age and time of sampling for the paediatric patients in the pharmacokinetic analysis of diclofenac and 4'-hydroxydiclofenac.

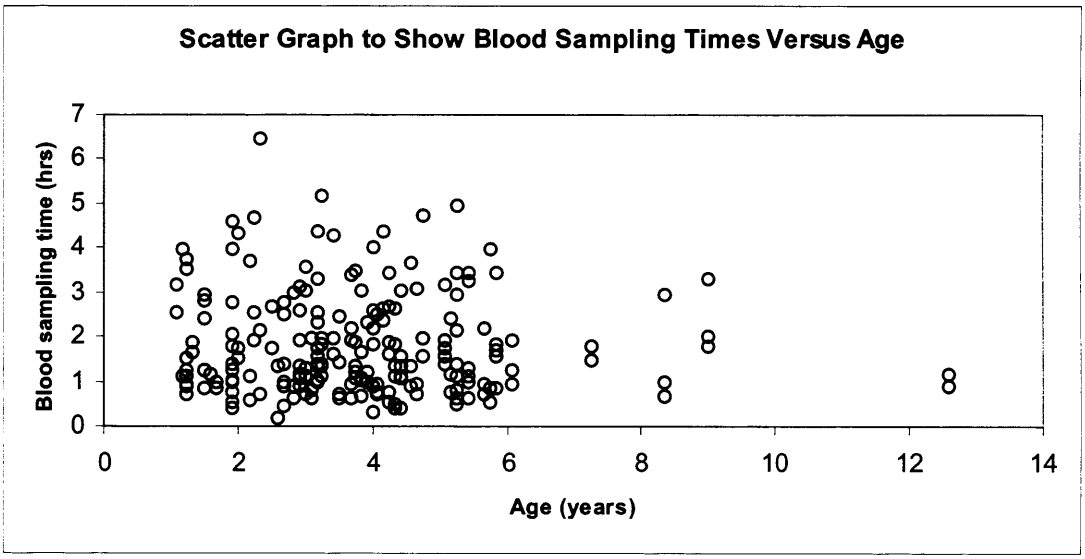
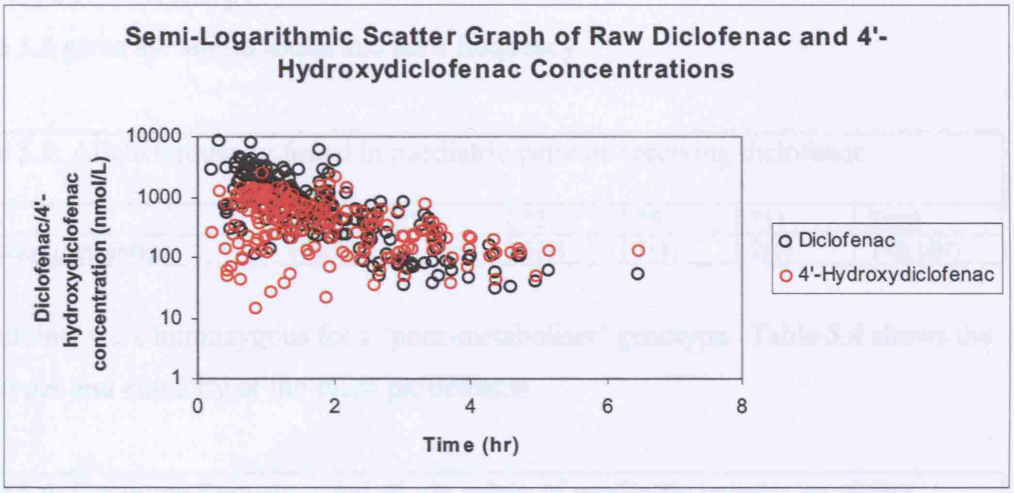


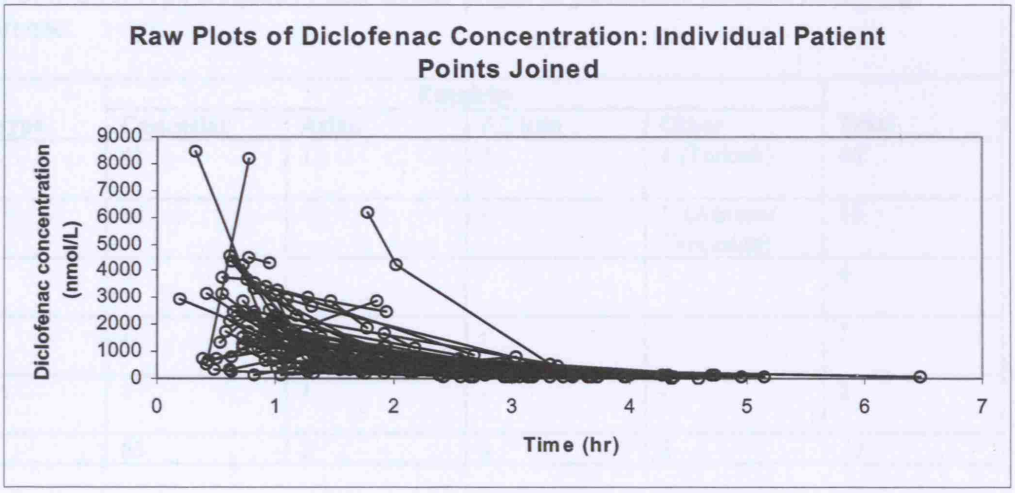
Figure 5.4: Relationship between age and sampling time for the paediatric patients. At each time point, measures of diclofenac and 4'-hydroxydiclofenac were made.

Figure 5.5 shows the observed diclofenac and 4'-hydroxydiclofenac concentrations.

a)



b)



c)

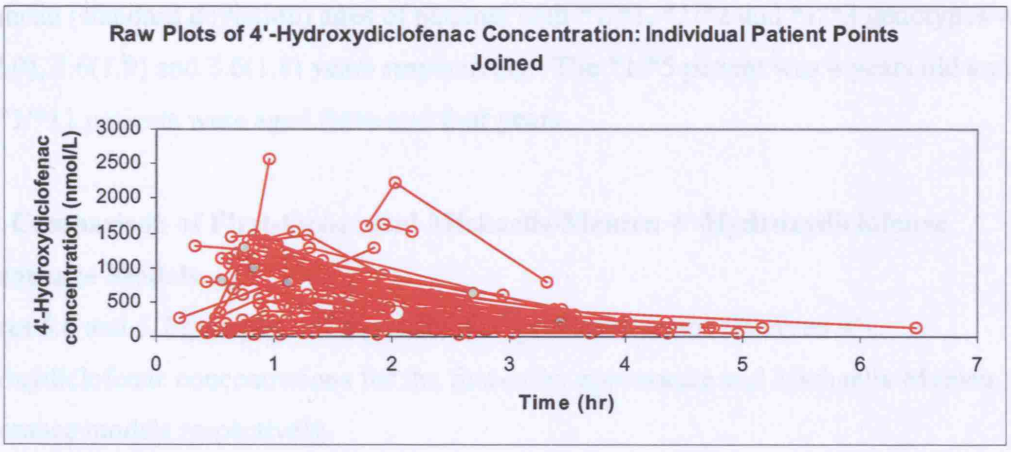


Figure 5.5: Plots of raw data: a) Diclofenac and 4'-hydroxydiclofenac. b) Diclofenac only. c) 4'-Hydroxydiclofenac only.

5.5.2 CYP2C9 Genotype

Table 5.3 gives the alleles found and their frequency.

Table 5.3: Allele frequency found in paediatric patients receiving diclofenac.

Allele	*1	*2	*3	*5	*11	Total
Frequency (percentage)	116(83)	15(11)	6(4)	1(1)	2(1)	140(100)

No patients were homozygous for a 'poor-metaboliser' genotype. Table 5.4 shows the genotypes and ethnicity of the study participants.

Table 5.4: Genotype frequency and ethnic origin of paediatric patients receiving diclofenac.

	Ethnicity				
Genotype	**Caucasian**	**Asian**	**African**	**Other**	**Total**
*1/*1	41	1	3	1 (Turkish)	46
*1/*2	14	-	-	1 (African/ Caucasian)	15
*1/*3	6	-	-	-	6
*1/*5	1	-	-	-	1
*1/*11	1	1	-	-	2
Total	63	2	3	2	70

The mean (standard deviation) ages of patients with *1/*1, *1/*2 and *1/*3 genotypes were 3.8(2.0), 3.6(1.9) and 3.6(1.8) years respectively. The *1/*5 patient was 4 years old and the two *1/*11 patients were aged three and four years.

5.5.3 Comparison of First-Order and Michaelis-Menten 4'-Hydroxydiclofenac Appearance Models

Figures 5.6 and 5.7 give plots of the individual predicted versus observed 4'-hydroxydiclofenac concentrations for the first-order appearance and Michaelis-Menten appearance models respectively.

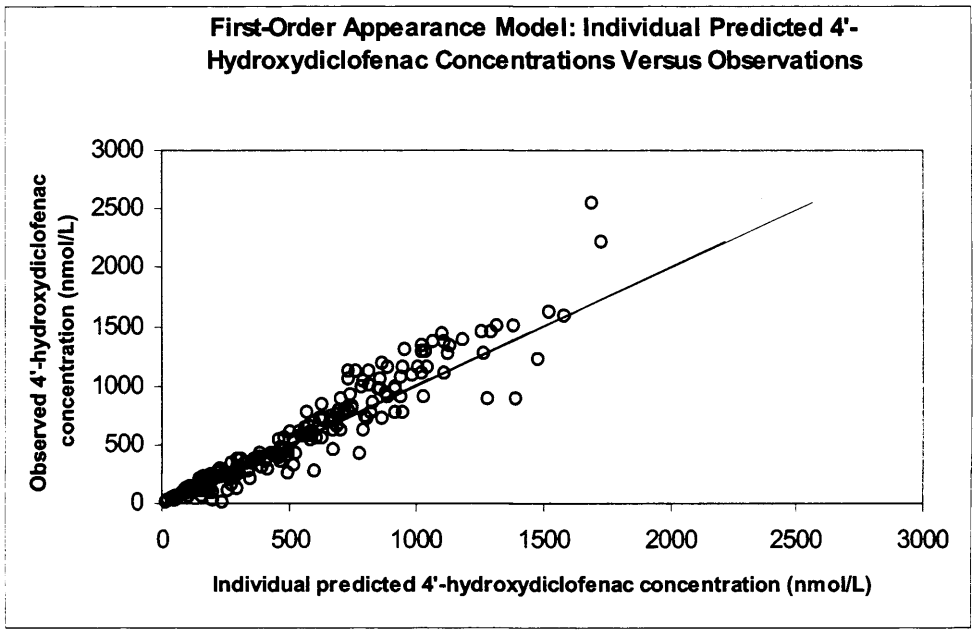


Figure 5.6: Scatter plot of individual predicted 4'-hydroxydiclofenac concentration versus observed concentration for the first-order appearance model.

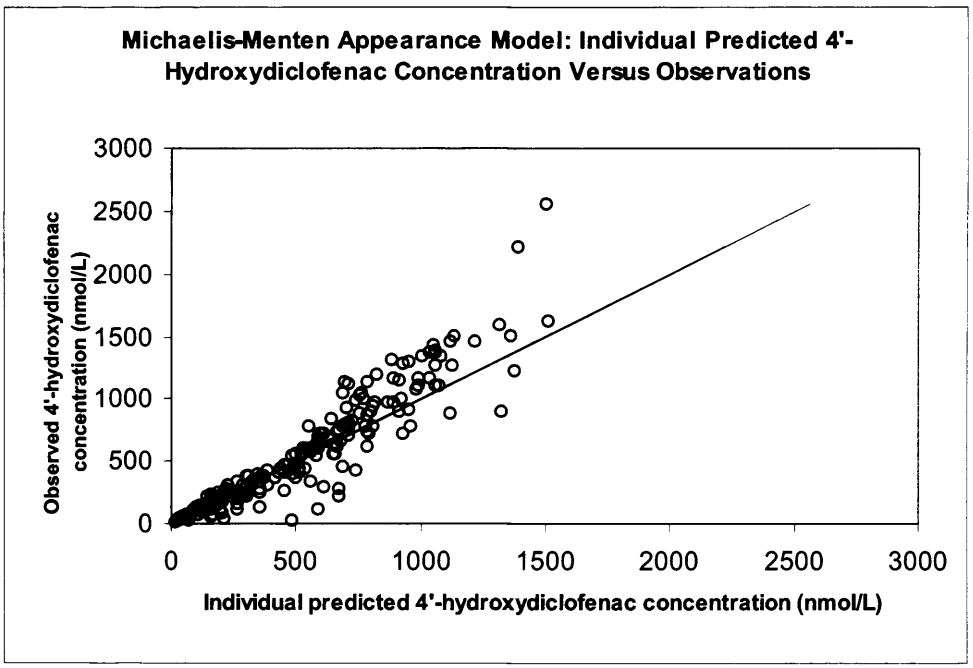


Figure 5.7: Scatter plot of individual predicted 4'-hydroxydiclofenac concentration versus observed concentration for the Michaelis-Menten appearance model.

Figures 5.8 and 5.9 give the weighted residual error versus time for the 4'-hydroxydiclofenac concentrations using the first-order and Michaelis-Menten appearance models respectively.

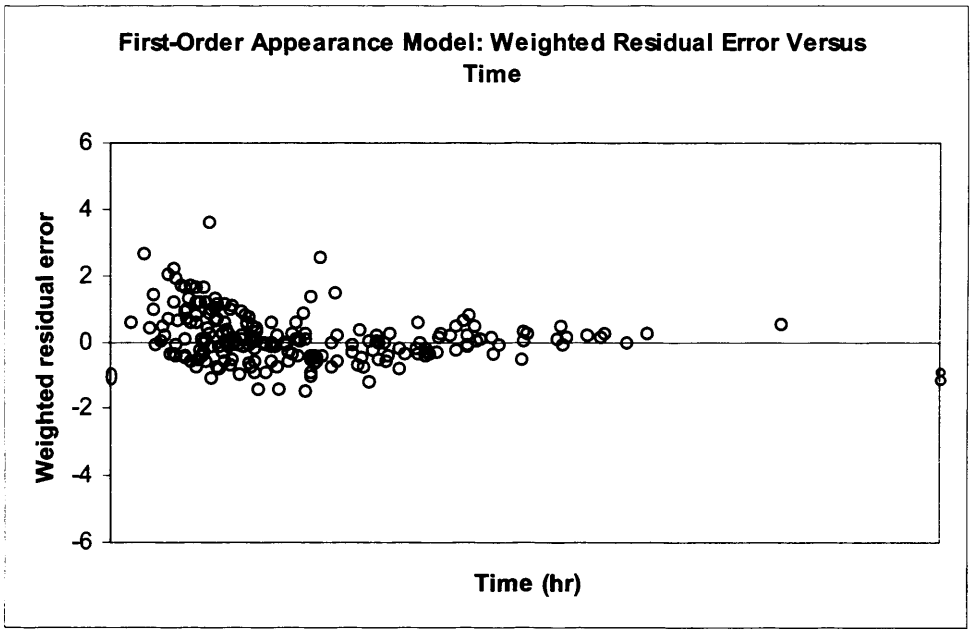


Figure 5.8: Scatter plot of weighted residual error versus time for the first-order 4'-hydroxydiclofenac appearance model.

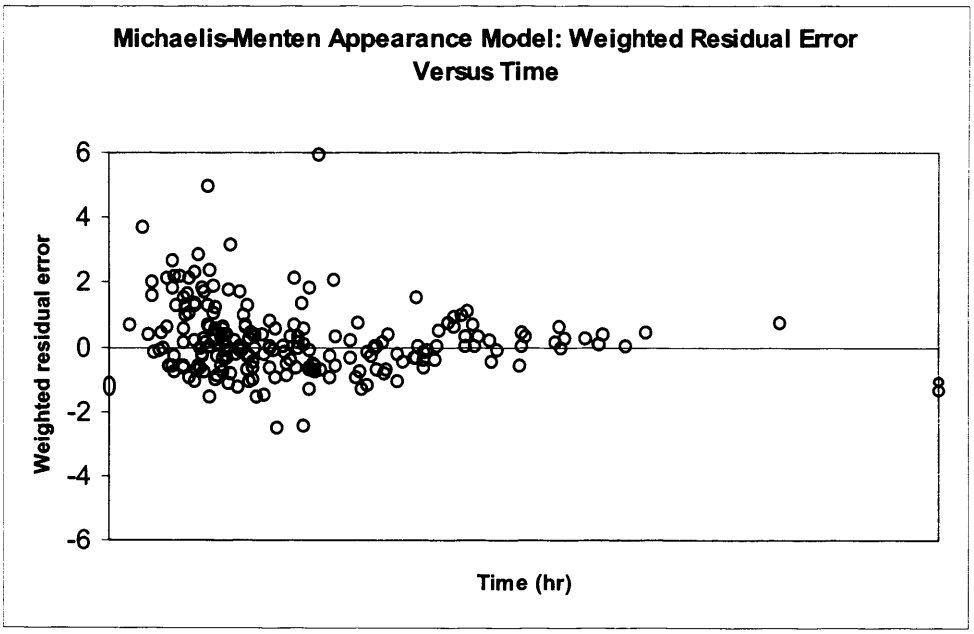


Figure 5.9: Scatter plot of weighted residual error versus time for the Michealis-Menten 4'-hydroxydiclofenac appearance model.

Figures 5.10 and 5.11 are visual predictive checks of the model's ability to predict 4'-hydroxydiclofenac concentrations for the first-order and Michaelis-Menten models respectively.

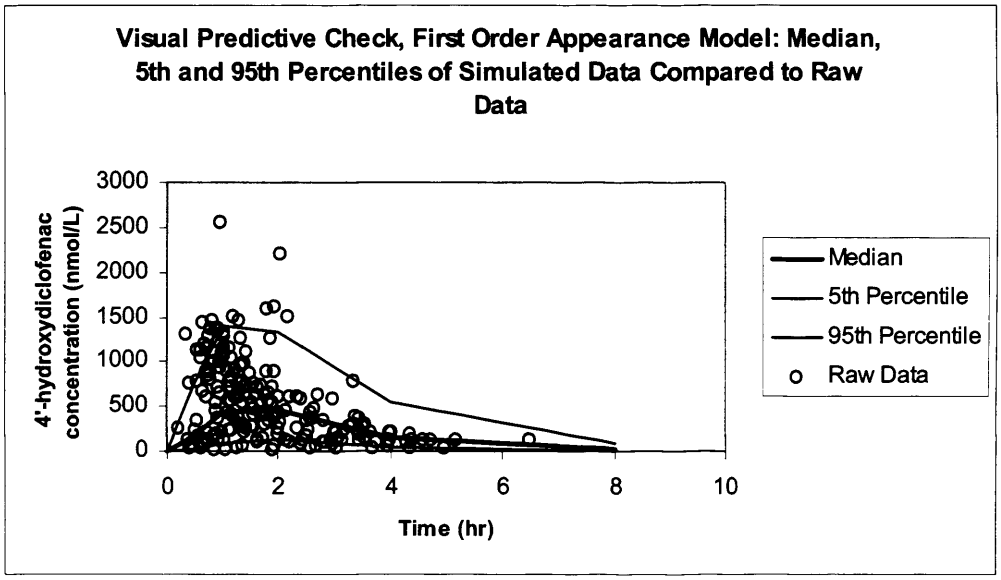


Figure 5.10: Visual predictive check of first-order appearance model: raw 4'-hydroxydiclofenac data superimposed on median, 5th and 95th percentiles of data simulated from model.

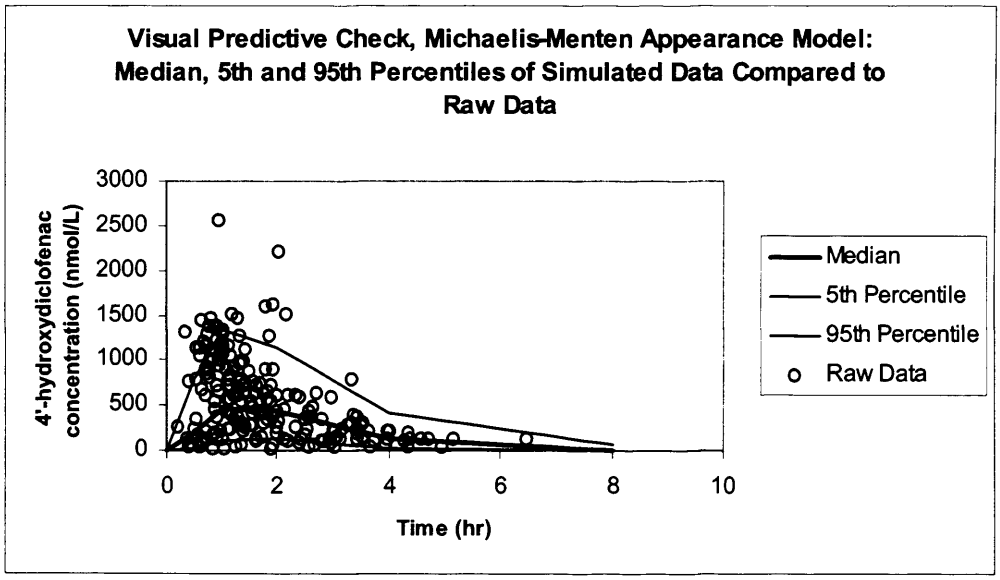


Figure 5.11: Visual predictive check of Michaelis-Menten appearance model: raw 4'-hydroxydiclofenac data superimposed on median, 5th and 95th percentiles of data simulated from model.

Figures 5.12 and 5.13 are visual predictive checks of the model's ability to predict diclofenac concentrations for the first-order and Michaelis-Menten models respectively.

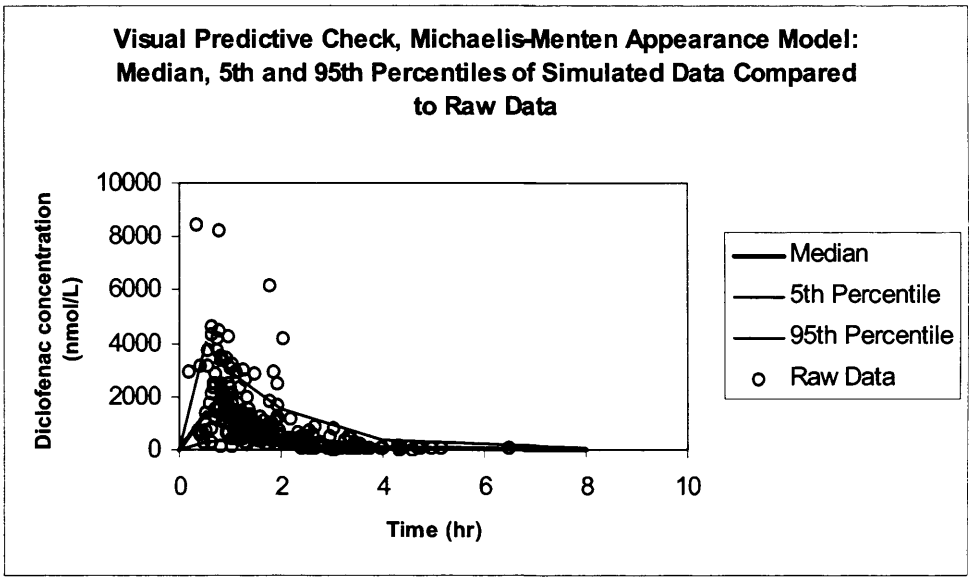


Figure 5.12: Visual predictive check of first-order appearance model: raw diclofenac data superimposed on median, 5th and 95th percentiles of data simulated from model.

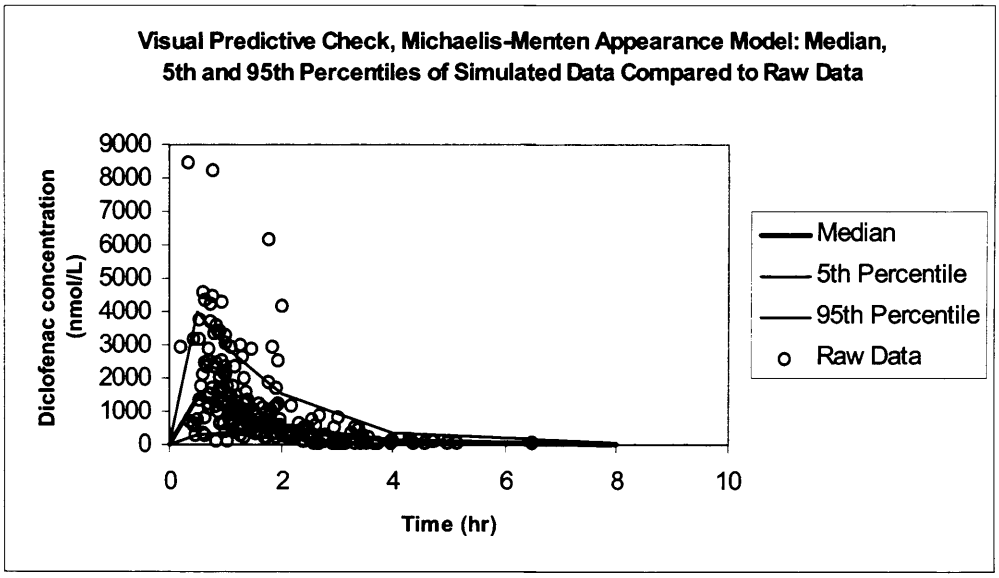


Figure 5.13: Visual predictive check of Michaelis-Menten appearance model: raw diclofenac data superimposed on median, 5th and 95th percentiles of data simulated from model.

Based on these plots (figures 5.6 to 5.13) the first-order appearance model was chosen.

5.5.4 Residual Error Model

As in the pharmacokinetic study (Chapter Two), a proportional residual error model best described the diclofenac concentrations. A proportional error model was also used to describe the residual error in 4'-hydroxydiclofenac predictions: figure 5.14 gives a plot of |IWRES| versus individual predicted 4'-hydroxydiclofenac concentrations from the first-order appearance model.

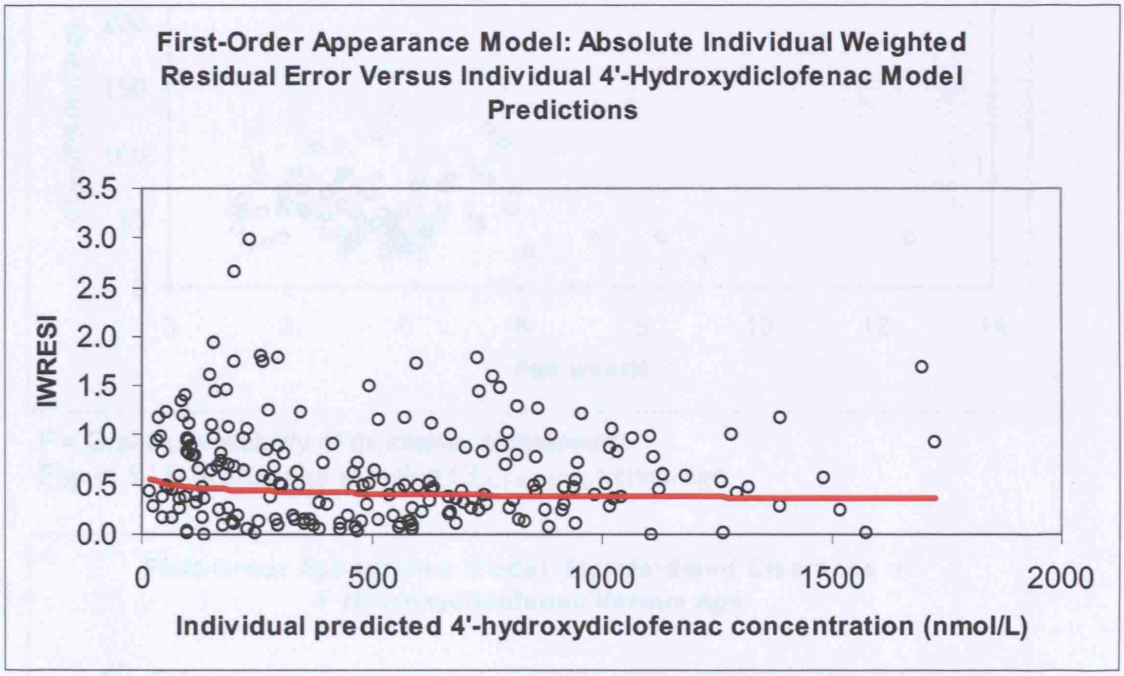
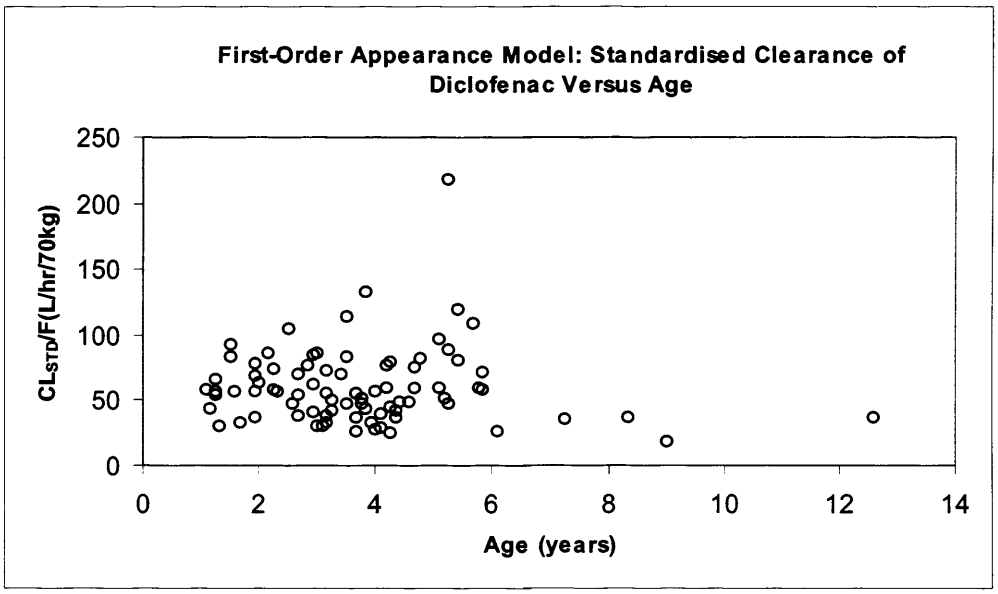


Figure 5.14: Absolute individual weighted residuals versus individual predicted 4'-hydroxydiclofenac concentrations for the first-order appearance model.

5.5.5 Influence of Age on Diclofenac and 4'-Hydroxydiclofenac Clearance Estimated from the First-Order Appearance Model

Standardised clearance, centred on 70kg according to the allometric size model (Meibohm B et al, 2005), plotted against age are given for diclofenac (CL_{STD}) and 4'-hydroxydiclofenac ($CL_{OF4OHSTD}$) are shown in figures 5.15 and 5.16.



F = Oral bioavailability of diclofenac suspension.
Figure 5.15: Scatter plot showing $CL_{OF4OHSTD}$ versus age.

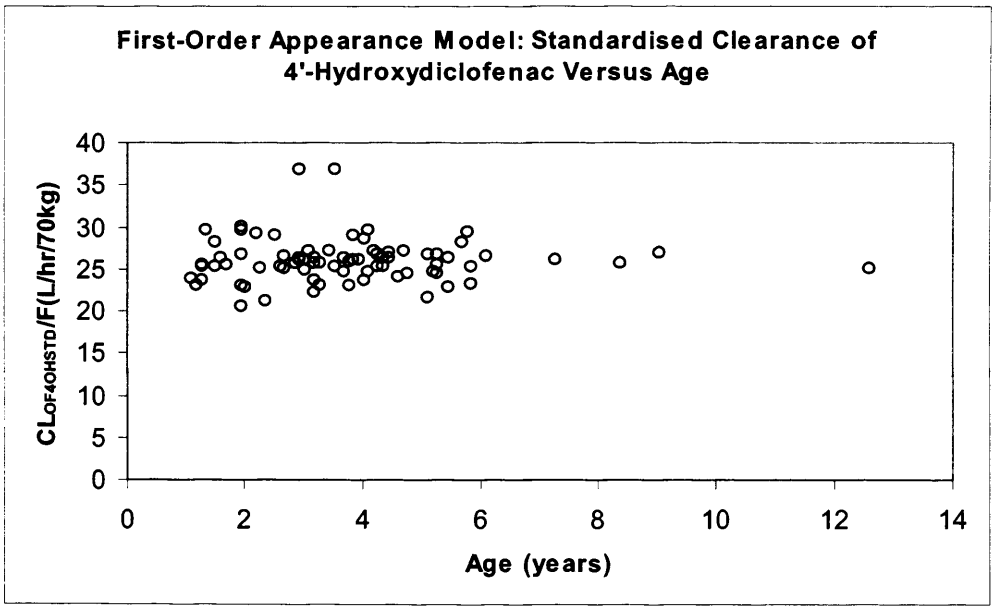


Figure 5.16: Scatter plot showing $CL_{OF4OHSTD}$ versus age.

5.5.6 Influence of Age on 4'-Hydroxydiclofenac Appearance Using the First-Order Model

Figure 5.17 shows the relationship between estimated diclofenac:4'-hydroxydiclofenac ratio with age.

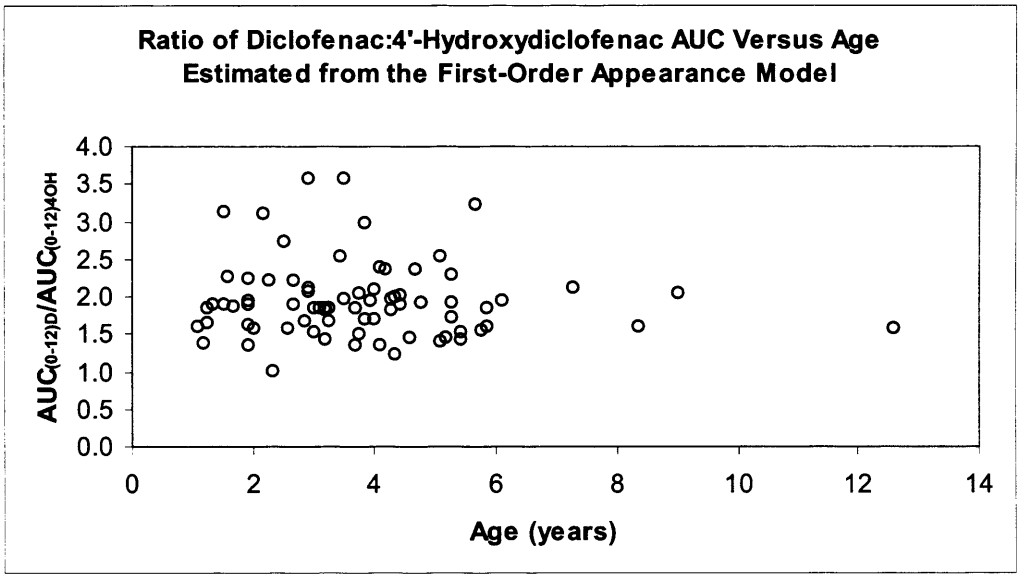


Figure 5.17: Diclofenac:4'-hydroxydiclofenac AUC ratio estimate from final model versus age.

5.5.7 Influence of CYP2C9 Genotype on 4'-Hydroxydiclofenac Appearance Using the First-Order Model

Table 5.5 gives the median diclofenac:4'-hydroxydiclofenac AUC ratio for each genotype.

Table 5.5: Comparison between median diclofenac:4'-hydroxydiclofenac AUC ratio and genotype.

CYP2C9 Genotype	Median $AUC_{(0\text{-}12)D}/AUC_{(0\text{-}12)4OH}$ (M-W test)	Median CL_{to4OH} (L/hr/70kg) (M-W test)
*1/*1	1.85	13.92
*1/*2	1.90 (p=0.67)	14.05 (p=0.74)
*1/*3	2.05 (p=0.20)	12.58 (p=0.12)
*1/*5	2.41 (p=0.28)	12.37 (p=0.36)
*1/*11	1.85 (p=0.94)	14.67 (p=0.51)

M-W test = Mann-Whitney non-parametric test for significance of difference between wild-type (*1/*1) value.

5.5.8 Final Parameter Estimates

Table 5.6 gives the final parameter estimates from the first-order 4'-hydroxydiclofenac appearance model.

Table 5.6: NONMEM parameter estimates from final first-order appearance model.

Fixed effects (θ)		Random effects (η)	
Parameter	**Estimate**	**Inter-individual variability (%)**	**Between occasion variability (%)**
MTT1 (hr)	0.68	13	-
N1	0.99	90	-
F1	0.67	23	-
$t_{1/2A1}$ (hr)	0.091	150	-
MTT2 (hr)	1.33	18	-
N2	40.60	144	-
$t_{1/2A2}$ (hr)	1.12	47	-
V_D/F (L/70kg)	5.15	56	133
CL/F (L/hr/70kg)	53.60	24	38
CL_{to4OH}/F (L/hr/70kg)	13.60	27	-
V_{Dof4OH}/F (L/70kg)	30 fixed	-	-
CL_{of4OH}/F (L/hr/70kg)	25.70	17	-
Residual variability (ε) (%):			
Adult diclofenac data:		26	
Paediatric diclofenac data:		20	
Paediatric 4'-hydroxydiclofenac data:		30	

- = Not estimated.

5.6 Discussion

The simultaneous modelling of diclofenac and 4'-hydroxydiclofenac proved an interesting challenge. This was largely because 4'-hydroxydiclofenac is formed during first-pass metabolism and from the small circulating free diclofenac fraction, yet the only model where all parameters were identifiable using serum levels was for 4'-hydroxydiclofenac to be formed from the central compartment (Venot A et al, 1987). For this reason it was decided to extend the model developed in the pharmacokinetic study (Chapter Two), as this model had already been found to explain and predict diclofenac concentrations well. Despite this restriction on the structural model, it was possible to optimise the rate of 4'-hydroxydiclofenac appearance by investigating first-order and Michaelis-Menten kinetics. From the diagnostic plots (figures 5.6 to 5.13) the first-order appearance model seemed to perform better than the Michaelis-Menten model. The predicted versus observed values

were closer in the first-order model (figures 5.6 and 5.7), and there was less residual error in predictions made by the first-order model (figures 5.8 and 5.9). The simulated visual predictive checks showed that the first-order model was slightly better at predicting the higher 4'-hydroxydiclofenac concentrations as can be seen by the number of data points above the 95th percentile of the simulated data (figure 5.10). There were 206 4'-hydroxydiclofenac data points in total, so the ideal model would have 11 raw data points (five percent) above the 95th percentile of simulated data. From figures 5.10 and 5.11 it can be seen that the first-order appearance model had approximately 15 points above the 95th percentile, whilst for the Michaelis-Menten model there were approximately 25 points. Both models seemed to perform equally well at predicting diclofenac concentrations (figures 5.12 and 5.13). The first-order appearance model was therefore chosen as it seemed better at predicting 4'-hydroxydiclofenac concentrations. Residual variability in 4'-hydroxydiclofenac predictions using the first-order appearance model was adequately described using a proportional error model. Evidence for this was seen in the trendless pattern of absolute individual weighted residual error versus individual 4'-hydroxydiclofenac predictions in figure 5.14.

Figure 5.5 shows that most 4'-hydroxydiclofenac observations were during the elimination phase. It is therefore likely that the main reason the first-order appearance model proved better than the Michaelis-Menten model, which reflects the actual rate of formation in the hepatocyte, was because relatively few observations were available during the 'appearance phase'. With little data, it was not possible to obtain estimates of a maximum rate of 4'-hydroxydiclofenac appearance (Vm_{app}), and therefore estimate Km_{app}, if indeed such parameters could be identified when the actual process of appearance was from both first-pass metabolism and transformation of any free circulating diclofenac. The first-order appearance model clearly fitted the data better, and table 5.6 shows that the estimated 4'-hydroxydiclofenac clearance of 25.7L/hr is similar to 10L/hr (Degen PH et al, 1988) and 27.5L/hr (Landsdorp D et al, 1990) reported elsewhere, which is a good indication that the model was able to predict 4'-hydroxydiclofenac pharmacokinetic parameters well.

Despite the first-order 4'-hydroxydiclofenac appearance being chosen as the final model, the diagnostic plots show it had some obvious flaws. Firstly, from the weighted residual

error versus time plot (figure 5.8) a clear trend in under-predictions can be seen at early time points. This indicates that actual 4'-hydroxydiclofenac concentration was higher than predicted, and is due to the fact that 4'-hydroxydiclofenac is produced during first-pass metabolism and so appears in the circulation at the same time as diclofenac, yet the model restricts 4'-hydroxydiclofenac appearance until after diclofenac appears. The second problem, which can be seen in the visual predictive check of 4'-hydroxydiclofenac (figure 5.10), is that it over-predicts 4'-hydroxydiclofenac concentrations between two and four hours, as can be seen by the large gap between the 95th percentile and observed data during this time. This is also as a result of the structural model only allowing 4'-hydroxydiclofenac appearance from the central compartment, when in fact it also appears during first-pass metabolism. High initial 4'-hydroxydiclofenac concentrations (from first-pass metabolism) force the model to assume clearance from the central compartment is more rapid than it actually is. At later time points after 4'-hydroxydiclofenac formed by first-pass metabolism has all entered the circulation, the model assumes a higher rate of appearance, leading to higher predicted concentrations than those observed. The result seen in the visual predictive check is an overestimation of 4'-hydroxydiclofenac AUC.

For the purposes of this study, which aimed to compare the diclofenac:4'-hydroxydiclofenac AUC ratio, the model overestimation of 4'-hydroxydiclofenac concentrations is only problematic if the degree of overestimation changes with body size or age. Figures 5.15 and 5.16 show the allometric size models successfully explained age-related differences in both diclofenac and 4'-hydroxydiclofenac clearance with body size. From this it can be assumed that the model performed consistently between subjects of different sizes, in that overestimations of 4'-hydroxydiclofenac concentrations did not change proportionally with size. Furthermore, figure 5.4 shows that sampling times were similar amongst all ages of patients, and so there is no theoretical reason that under-predictions at early time points and over-predictions at later time points caused by the structural model flaws should differ between age groups. It should therefore be reasonable to use the first-order appearance model to compare the AUC ratio of diclofenac:4'-hydroxydiclofenac with age as an indicator of CYP2C9 expression. By showing that the diclofenac-4'-hydroxydiclofenac ratio does not appear to change with age, figure 5.17

confirms previous *in vitro* findings (Koukouritaki SB et al, 2004) that indicate CYP2C9 expression should be mature before one year of age.

A potential problem with using AUC ratio of drug and metabolite in *in vivo* studies is that this ratio can be affected by changes in renal clearance of the metabolite as shown in equation 5.6 (Tucker GT et al, 1998):

$$\frac{AUC_{DRUG}}{AUC_{METAB}} \approx \frac{fu_{METAB} \times CLUR_{METAB}}{CLU_{TOMETAB}} \qquad \text{Equation 5.6}$$

Where:

fu_{METAB} = Metabolite fraction unbound.
$CLUR_{METAB}$ = Renal clearance of metabolite fraction unbound.
$CLU_{TOMETAB}$ = Intrinsic formation clearance of unbound drug to metabolite.

As renal function is probably mature by age one year (Kearns G et al, 2003) and $CL_{OF4OHSTD}$ does not change with age (figure 5.16), this particular confounding factor does not appear to affect the present study. It will be important for future studies in younger age groups recognise the need for renal function maturation to be accounted for when comparing drug and metabolite AUC ratio. The degree to which 4'-hydroxydiclofenac can be liberated from CYP2C8-catalysed 4'-hydroxylation of diclofenac glucuronide (Tang W, 2003) in the tissues is unknown, and although amounts are unlikely to be as significant as in urine, it remains a possible confounding factor of this study.

The second part of the study was to investigate the CYP2C9 genotype of the subjects, as this was a potential confounding factor in diclofenac 4'-hydroxylation. As can be seen in section 5.5.1, the mean ages of patients with different genotypes were similar, and so no confounding by genotype on the analyses of age versus 4'-hydroxydiclofenac appearance is expected. Most patients in this study were Caucasians and the distribution of *1/*1, *1/*2 and *1/*3 genotypes of 65.1, 22.2 and 9.5 percent respectively (table 5.4) was similar to that seen in several other studies in this ethnic group (Lee CR et al, 2002). A Caucasian patient in this study possessed a *5 allele which was only thought to be expressed in subjects of African origin (Dickman LJ et al, 2001). This exemplifies the need for the new field of 'personalised medicine' to continue to move away from seeking inter-ethnic

differences, and more towards recognising individual genetic differences (Suarez-Kurtz G, 2006). Although the proportion of genotypes for CYP enzymes may differ amongst populations of different ethnic origin, it is clear that ethnicity alone is too crude a measure to predict possible drug response (Sistonen J et al, 2007).

No patients in this study were homozygous for a potentially poor metaboliser genotype, and table 5.5 shows that there was a trend for an increase in the diclofenac:4'-hydroxydiclofenac AUC ratio, and decrease in standardised CL_{TO4OH}, in those with a single defective allele, although these were not statistically significant. This trend was pronounced in particular in patients with the *1/*3 genotype, reflecting what would be expected from *in vitro* data (table 5.1). Interestingly, a previous adult study found a mean diclofenac:4'-hydroxydiclofenac AUC ratio of 1.9 for *1/*1 patients and 2.2 for *1/*3 patients (Shimamoto J et al, 2000), which are similar to the values of 1.85 and 2.05 found here (table 5.5). This was the first *in vivo* study to look at the influence of *5 and *11 polymorphisms on diclofenac 4'-hydroxylation *in vivo*. Whilst the two patients with the *1/*11 genotypes had similar diclofenac:4'-hydroxydiclofenac AUC ratios and CL_{TO4OH}.to wild-type, the patient with *1/*5 genotype had higher than the median diclofenac:4'-hydroxydiclofenac AUC ratio for either wild-type or *1/*3 patients, and lower CL_{TO4OH}. This may suggest that as with *in vitro* findings (table 5.1), the *5 polymorphism may have the most effect on CYP2C9 activity.

Although this study was not designed to test the effect of genotype on diclofenac 4'-hydroxylation, several previous adult studies have addressed this question. Diclofenac has largely been discounted as a useful probe for determining CYP2C9 genotype because most studies did not find a significant difference in 4'-hydroxydiclofenac formation and CYP2C9 genotype (Aithal GP et al, 2000, Kirchheiner J et al, 2002, Morin S et al, 2001, Shimamoto J et al, 2000, Yasar U et al, 2001). However, the largest study to-date with 102 participants did find reduced diclofenac 4'-hydroxylation in patients with *1/*3 and *2/*3 genotypes, and as can be seen in table 5.5, there appeared to be a trend towards patients with *1/*3 genotypes having reduced capacity for diclofenac 4'-hydroxylation in the present study.

The fact that diclofenac is not an ideal probe in investigating CYP2C9 genotype does not necessarily mean it has limited value in predicting CYP2C9 expression. As can be seen in table 5.1, alterations in enzyme composition often lead to decreased *in vitro* Km values (a measure of enzyme affinity for the substrate), but Vm tends to be fairly similar to the wild-type (Takanashi K et al, 2000). Where there is decreased enzyme concentration due to lack of expression, a decrease in Vm would be expected (Rowland M & Tozer TN, 1994), which would have a greater impact than Km decreases in situations where substrate concentration is relatively high, for example during first-pass metabolism. For this reason, the fact that diclofenac is not a good *in vivo* probe for CYP2C9 genotype, does not necessarily mean it is not useful in determining CYP2C9 expression. To confirm whether CYP2C9 expression follows the same pattern of the *in vitro* findings (Koukouritaki SB et al, 2004), an extension of this study in neonates and young infants, where the expression of CYP2C9 would be expected to differ between birth and five months of age, is required. As diclofenac is generally not used in this group of patients, future studies using other CYP2C9 probes such as tolbutamide methydroxylation, S-warfarin 7'-hydroxylation or phenytion 4'-hydroxylation (Lee CR et al, 2002) are required in neonates and infants to confirm the age at which expression can be considered adult equivalent. Although constraints due to surgery were placed on the sampling times in the present study, such future studies should aim to utilise optimal design strategies (Roy A & Ette EI, 2005) to maximise the informativeness of sparse sampling usually available in paediatric patients.

5.7 Conclusions

Although the simultaneous compartmental modelling of diclofenac and 4'-hydroxydiclofenac proved difficult, it is reasonable to expect that proportion of over-estimation of 4'-hydroxydiclofenac concentrations, and therefore AUC, by the final model should not change with age. In comparing the ratio of diclofenac:4'-hydroxydiclofenac AUC, this study has apparently confirmed *in vitro* findings that CYP2C9 expression should be adult-equivalent by one year of age (Koukouritaki SB et al, 2003). The *in vitro* data suggests that hepatic expression of CYP2C9 is fully developed by five months of age, and so future *in vivo* studies in neonates and infants are required.

As with previous studies seeking a relationship between 4'-hydroxydiclofenac formation and CYP2C9 genotype, this study failed to find a statistically significant link, although there seemed to be a trend with decreased 4'-hydroxydiclofenac formation especially amongst *1/*3 patients. This study was also the first *in vivo* study to investigate the influence of the *5 allele, and although it was only found in a single patient, the fact that the diclofenac:4'-hydroxydiclofenac AUC ratio was higher than the median for *1/*3 patients suggests this polymorphism may have a larger impact on *in vivo* metabolism.

Whilst it is clear that genetic polymorphisms and developmental differences in CYP2C9 expression have some impact on pharmacokinetic variability, further research is required to determine whether it has relevance for dosing. Even for a narrow therapeutic index drug such as warfarin, which is metabolised by CYP2C9, it may be some time before knowing a patient's genotype will improve dosing in the clinic. This is exemplified by a recent study on the influence of CYP2C9 and vitamin K epoxide reductase complex subunit 1 (VKORC1) genotype on warfarin dosing (Takanashi H et al, 2006). The authors state: "dosing requirements for a similar degree of anticoagulation varies across populations and appears to be related to racial ancestry". In the demographics table describing patients entering the study, the mean warfarin daily dose for African-American, Caucasian and Japanese patients was 5.3, 4.7 and 3.5mg respectively. The same table also gives the mean weight for African-American, Caucasian and Japanese patients, which was 89.5, 73.7 and 56.5kg respectively (Takanashi H et al, 2006). Dividing dose by body weight shows that the mean daily warfarin dose, regardless of ethnicity, was 0.6mg/kg. This example shows the importance of delineating known factors that affect pharmacokinetics, such as body size, before trying to explain inter-individual variability such as that arising from genetic polymorphisms or enzyme expression.

Chapter SIX: Overall Discussion and Conclusions

6.1 Summary and Significance of Findings

Published clinical studies on diclofenac for acute pain in children have used doses ranging from 0.5 to 2.5mg/kg (Tay CLM & Tan S, 2002, McGowan PR et al, 1998) and there is currently still confusion amongst paediatric anaesthetists as to the optimum dose (Eustace N & O'Hare B, 2007). The pharmacokinetic study carried out in this thesis has answered the question on optimum dosing: that 1mg/kg of diclofenac for children aged one to 12 years achieves a similar exposure to 50mg (the optimum dose) in adults. The significance of this finding is that when the results are published and disseminated to paediatric anaesthetists, it will hopefully lead to children receiving optimum analgesia from diclofenac (1mg/kg), without unnecessary overdosing and potentially increased risk of adverse effects.

The incidence of serious adverse reactions of diclofenac appeared to be rare (less than 0.1 percent), but spontaneous reports did show that children appeared to suffer similar types of serious adverse drug reactions as those seen in adults. The other two key findings of the safety studies were that diclofenac does not appear to increase the risk of bleeding when given for peri-operative analgesia, and that patients receiving diclofenac seem to suffer less nausea and vomiting than those treated with only non-NSAID analgesics. The message to paediatric anaesthetists and other health professionals treating children with acute pain is that diclofenac appears to have a similar safety profile to that in adults, patients may have less nausea and vomiting if diclofenac is used as part of the peri-operative analgesic regimen, and that peri-operative bleeding is not increased with the use of diclofenac.

During the planning of the safety study it was hoped that in addition to recording adverse events for the purpose of pattern recognition, as highlighted by the practolol disaster (Abraham J & Davis C, 2006), a good understanding of the incidence of common adverse reactions to diclofenac would be gleaned. Unfortunately the observational safety study was less successful than hoped at identifying which adverse events were caused by diclofenac due to the multiple interventions made in the peri-operative period. Although this was partially due to the fact that most patients only received a single dose of diclofenac in the

operating theatre (if post-operative NSAIDs were required ibuprofen was often given) coinciding with single doses of several other analgesics, anaesthetics and antibiotics, the main problem was a lack of objectivity in the causality assessment process. For non-specific reactions such as minor gastrointestinal upset, this study has shown that even with rigorous causality assessment and expert panel review, ascribing an effect to one of several possible drugs is not usually possible. It is for this reason that comparative studies are required, and as it is unlikely that such studies investigating only adverse effects will be instigated, it is crucial that efficacy studies systematically monitor and report adverse events. This will allow future treatments choices to be made on a rational basis accounting for both efficacy and adverse reactions.

The final question, which unfortunately the clinical safety study and systematic review were unable to answer, was on the prevalence of diclofenac-induced bronchospasm in asthmatic children. There seemed to be a clear diversity of opinion amongst paediatric anaesthetists as to whether diclofenac (or other NSAIDs) should be with-held from asthmatic patients, but as most clinical studies either excluded asthmatics or did not record the prevalence of asthma in the patients receiving diclofenac, ascertaining the incidence of diclofenac-induced bronchospasm was not possible. The fact that some anaesthetists seem to use diclofenac regardless of whether a patient is asthmatic, probably rationalising that NSAIDs are the most effective simple analgesic for acute pain in children (Clark E et al, 2007) and bronchospasm would be managed well in the hospital setting should it occur, means that if diclofenac-induced bronchospasm is indeed common, there should be a large proportion of case reports describing the phenomenon. As it is not the case that diclofenac-induced bronchospasm is commonly reported, the balance of probability lies with it being a rare event, a supposition that needs to be confirmed, possibly with a large observational-type study on asthmatic children receiving diclofenac.

Recently introduced European legislation allowing manufacturers to apply for a Paediatric Use Marketing Authorisation (PUMA) for off-patent drugs which are used 'off-label' in children (Stephenson T, 2006) could mean that the information on dosing and safety generated in this thesis will aid diclofenac to become licensed for acute pain in children. It is currently unclear whether this legislation will indeed stimulate the pharmaceutical

industry into developing and licensing new paediatric formulations. This is because even with the eight-year patent protection offered by the PUMA, the paediatric market is still relatively small, meaning such ventures may not be very profitable. For this reason, the most important aspect of the dosing and safety information generated in this thesis, at least once the findings are published, is that more information on diclofenac will be available to healthcare professionals involved in treating children with acute pain. The importance of this information being available is that diclofenac is used 'off-label' for acute pain in children, meaning that if harm should occur to the patient the manufacturer cannot be held liable. This means that prescribers of medicines outside the terms of a product license must take legal responsibility should the patient come to any resulting harm. The Bolam Principle is a legal ruling that supports prescribing outside the terms of a product license in that provided sufficient evidence or expert medical opinion exists that 'off-label' or unlicensed use of a medicine is warranted, prescribers should not be prosecuted due to any resulting harm to the patient (Stephenson T, 2006). The findings of this study provide such evidence and should assure prescribers treating children with acute pain that diclofenac 1mg/kg is a reasonable choice with a low risk of inducing a serious adverse reaction.

Findings from a previous *in vitro* study that CYP2C9 expression was adult-equivalent by five months of age (Koukouritaki SB et al, 2004) were partially confirmed in the *in vivo* study on diclofenac 4'-hydroxylation, which showed no age-related difference between children aged one to 12 years. This finding can now be used for such applications as the development of physiologically-based pharmacokinetic models which attempt to predict paediatric pharmacokinetics (Edgington AN et al, 2006, Johnson TN et al, 2006). Indeed, anyone wishing to predict the pharmacokinetics of a CYP2C9 substrate with a view to designing a dosing regimen in children should not expect enzyme expression to change after one year of age. The second main finding of this study was that CYP2C9 genotype for heterozygous poor-metabolisers did not have a significant influence on 4'-hydroxydiclofenac formation, but more subjects with *5 and *11 alleles would have been required to test their influence. The main reason for undertaking genotyping was that it was a potential confounding factor in the investigation of CYP2C9 expression: had one age group of patients contained a higher proportion of poor metaboliser genotypes, it may have led to a false conclusion about CYP2C9 developmental expression. The fact there was no

trend in the distribution of genotypes with age, and that genotype did not have a significant influence on 4'-hydroxydiclofenac formation, should reinforce confidence in the finding that CYP2C9 expression is adult equivalent by one year of age.

6.2 Recommendations for Undertaking Clinical Drug Studies in Children

The studies reported here have shown that recruiting paediatric patients to take part in clinical drug studies is certainly feasible, and can provide valuable answers to clinical questions. Experience gathered on the problems encountered and the aspects that worked well in carrying out drug studies in children will be outlined in this section.

Both clinical studies required ethical approval, which entailed filling out lengthy forms for the ethics committee, research and development office (for study costing), data protection, and risk assessment. In addition, a study protocol, information leaflets for both the patient and parents, and general practitioner letter had to be submitted. This process took approximately two to three months for each study and it is important that this time is factored in when planning such studies. The main problem encountered during the ethical approval process centred on the patient information leaflets. For the pharmacokinetic study, the ethics committee deemed it necessary to design a separate information sheet for patients older than eight years, and eight years and younger (Appendix 7.3). The idea to use a cartoon format in order to keep the younger age-group leaflet as simple as possible was taken from a colleague's leaflet for a haematology study. The advice on patient information leaflets therefore is to be aware that what may seem a trivial factor (it is doubtful that many of the patients under four years were read the leaflet by their parents) can prove difficult to design. This may delay the approval process, so it is advisable to draw inspiration from leaflets already approved for other studies.

In addition to ethical approval, authorisation from the medicines regulator (in this case the MHRA) is required through the awarding of a Clinical Trials Authorisation (CTA) certificate. This involves providing the regulator with detailed pre-clinical and clinical information on the drug and can also be time consuming. For the studies presented in this thesis the sponsor (Rosemont Pharmaceuticals Ltd.) completed the MHRA application, which luckily was not as onerous as the current CTA system as the regulations in place at

the time allowed an exemption from the full authorisation for investigator-led studies (Clinical Trial Exemption certificate). The advice on regulatory approval is to seek the help of the drug manufacturer, as they will have ready access to the detailed information, especially pre-clinical data such as animal toxicology that is required.

When conducting clinical studies in a hospital it is important that health professionals involved in the care of the patients have a good understanding of what the study entails, and also why the study is being carried out. During the pilot data collection for the observational safety study, one of the consultant surgeons asked for more information about why the study was necessary as diclofenac had been used for many years. When it was explained that diclofenac was being used 'off-label', the consultant became concerned that perhaps this would worry the parents unduly. By explaining the meaning of unlicensed and 'off-label' and that the very purpose of the study was to provide more information to prescribers using such medicines, the fears of this particular consultant were allayed. However, this incident highlighted a clear need to provide more information on the proposed study, so presentations to staff on all wards where patients were recruited were made, not only to explain the logistics, but to explain its rationale and to field any questions. This approach was then used for the pharmacokinetic study where a presentation to the anaesthetists in the planning stage meant that they understood why the study was being undertaken, which undoubtedly contributed to their willingness to help with logistics such as prescribing pre-operative diclofenac and blood sampling in the operating theatre. The advice on making the study run smoothly is perhaps obvious (but sometimes difficult to achieve due to the large number of people involved in the care of hospitalised patients): ensure that the purpose and logistics of the study are communicated to all relevant staff members before starting the recruitment phase.

The recruitment of patients to the observational safety study initially proved much more difficult than originally envisaged. The main reason for this was that the ethics committee insisted parents had at least 24 hours to think about whether to take part before deciding. To achieve prospective recruitment, this meant that parents and patients had to be approached prior to admission, which was done either in the pre-admission clinic or by post with a pre-paid envelope in which the consent form could be returned. The problem with

this strategy was that many patients did not have a routine pre-admission appointment (the decision to perform surgery being made at a standard out-patient appointment and blood tests and physical examination being performed on admission for surgery) and the return rate of completed consent forms was very low. The solution devised was to post out information leaflets to give parents and patients time to think about the study, and then to approach them in person on the morning of admission. This improved the recruitment rate, probably because it afforded parents and patients the opportunity to ask questions at the time of signing the consent form, and did not rely on them having to remember to post the form back. A similar strategy was used in the pharmacokinetic study whereby parents were telephoned in advance of the admission to inform them about the study, and then patients were recruited on the day of admission. This proved very effective as the recruitment rate of 77 percent shows. The advice on patient recruitment is that it is important to give patients and parents time to think about the study, either by telephoning or sending an information leaflet in advance, but the final decision should be made face-to-face with the researcher so that any questions can be addressed, and to avoid the problem of losing those who intend to participate but forget to return the consent form.

The timing of dosing and blood sampling in the pharmacokinetic study was largely dictated by when the patient was due to go to the operating theatre and how long the operation would take. These were uncontrollable factors from a research perspective, but it was important that those variables that could be controlled were. For this reason the researcher was present when all doses were administered to check the correct volume was given and that the patient swallowed the dose, and also for most blood sampling to record the time taken. It was not possible to be present at every blood sampling time as two patients per theatre list were sometimes recruited, meaning that the researcher was in theatre collecting samples from one patient whilst another would have returned to the ward and be having the third sample. For this reason each patient was given a digital watch and nurses assisting with the study were able to note the time to the nearest minute of blood sampling if the researcher was not present. The advice that this approach highlights is that in clinical pharmacokinetic studies where some factors may be out of the control of the investigator, it is crucial to maintain scientific control to minimise the variability in the measurements of dose, dosing time and sampling time.

The final recommendation is that when clinical studies are carried out in children, it is important to maximise the information gained. For example, the pharmacokinetic study aimed to ascertain the dose of diclofenac in children, but samples were also analysed for 4'-hydroxydiclofenac and genotyping. No extra interventions were made to obtain these samples, as they were drawn at the same time as the pharmacokinetic samples, but a study on the ontogeny of CYP2C9 and the influence of genotype on metabolism of diclofenac to 4'-hydroxydiclofenc were possible. With new legislation meaning that increasing numbers of dosing studies will be carried out in children, the question of whether it is ethical to only investigate pharmacokinetics when there is a possibility to also study areas such as developmental pharmacology and pharmacokinetic/pharmacodynamic relationships, must be carefully considered.

6.3 Areas for Future Research

The main question left unanswered by the safety studies was on the prevalence of diclofenac-induced bronchospasm in asthmatic children. From the small number of asthmatic children recruited and a published bronchoprovocation challenge in asthmatic children given diclofenac (Short JA et al, 2000) it was concluded that diclofenac-induced bronchospasm occurred in less than 2.7 percent of asthmatic children. In order to ascertain the actual prevalence of diclofenac-induced bronchospasm in to allow a more informed decision as to whether these patients should be denied what is an effective analgesic, a large observational study on asthmatic children receiving diclofenac is required.

The studies conducted in this thesis show that diclofenac for acute pain in children seems at least as safe as in adults (in that serious adverse reactions do not appear to be more common), and recommends a dose of 1mg/kg. Currently patients aged less than one year tend not to be prescribed diclofenac, and this is presumably because of concerns about renal function maturation and the risk of acute renal failure. As with asthmatic children, it appears that neonates and young infants are denied a potentially effective analgesic due to a perceived risk; future research to investigate this potential for NSAIDs to cause renal dysfunction in young infants and to assess efficacy and dosing in these patients is warranted.

Descriptive pharmacokinetic studies on diclofenac for acute pain in children have been reported for intravenous administration (Korpela R & Olkkola KT, 1990), enteric-coated tablets (Romsing J et al, 2001) and suppositories (Murphy DB et al, 2000, van der Marel CD et al, 2004), but none sought to recommend a dose. A pooled pharmacokinetic meta-analysis of these studies, in addition to the data on diclofenac oral suspension, could be undertaken to ascertain the relative bioavailability and dose information for each formulation.

No difference was seen in the formation of 4'-hydroxydiclofenac in children aged one to 12 years, suggesting that the developmental expression of CYP2C9 is complete by at least one year. What now remains is to undertake a similar investigation in neonates and young infants to confirm whether five months, the age at which an *in vitro* study has ascertained hepatic CYP2C9 expression is adult-equivalent (Koukouritaki SB et al, 2004), is a reasonable assumption. Such a study would need to use a different probe drug as diclofenac is rarely used in children under six months of age due to concerns over developing renal function. Further *in vivo* investigation on the effect of the *5 allele of CYP2C9 is also required as it potentially has less metabolic activity than *3 (Dickman LJ et al, 2001).

The final and most important area for future research centres on the *a priori* use of allometric $w^{3/4}$ scaling of clearance terms in empirical pharmacokinetic studies such as the one performed on diclofenac. Whilst basal metabolic rate scales with $w^{3/4}$ across species, whether it scales with $w^{3/4}$ within a species during development is currently unknown. Furthermore, research is needed on why and whether drug clearance should also follow this $w^{3/4}$ pattern.

> "*Ma guai a chi cede alla tentazione di scambiare una ipotesi elegante con una certezza.*" ("But there is trouble for those who surrender to the temptation of mistaking an elegant hypothesis for a certainty.")
> (Levi P, 1975).

At the moment, scaling drug clearance with $w^{3/4}$ provides an elegant hypothesis, and whilst it seemed to adequately predict diclofenac clearance in children older than one year, it often fails to predict drug clearance in neonates (Allegaert K et al, 2005). Physiologically-based pharmacokinetic models, which attempt to account for maturational processes, may predict clearance and dosage in neonates and infants better than allometric models (Bjorkman S, 2006), but is there an as yet unrecognised allometric scale to predict doses in these patients? Developmental pharmacology research has focussed on how groups of patients differ, citing the adage 'children are not small adults'. There are physiological differences between children and adults, as discussed in detail in Chapter One, but there are also similarities: a pump (heart) distributes blood through a branching network of tubes (circulation), through a filter (liver), and a drain (kidneys) removes impurities. These similarities need to be found if a dose is to be recommended for a population. Just like the elephant is similar to the mouse in that basal metabolic rate scales with $w^{3/4}$, is there a relationship linking age, weight and basal metabolic rate in the baby and the adult, and could it be used to delineate expected maturation from other pharmacokinetic variability in drug handling? Further research on the scaling of doses between individuals of different sizes is required, both to validate existing theories and to generate new hypotheses.

6.4 Conclusions

The key findings of the studies undertaken here are: the optimum dose of diclofenac for acute pain in children aged one to 12 years is 1mg/kg; children seem to suffer similar types of adverse reactions to adults treated with diclofenac, and the incidence of serious adverse reactions is no more common in children than adults; giving diclofenac as part of the peri-operative analgesic regimen seems to reduce the incidence of nausea and vomiting by about 40 percent; diclofenac does not increase the incidence of peri-operative bleeding; and CYP2C9 expression is adult-equivalent by one year of age.

The information on diclofenac dosing and safety presented here has answered important questions for health professionals currently treating children with acute pain. By suggesting an optimum dose, and showing adverse reactions are of a similar type and seemingly no more prevalent than in adults, these results will aid the decision-making process on whether and how to use diclofenac for children with acute pain. Further

research on the incidence of bronchospasm in patients with asthma is warranted, and on dosing and safety in patients younger than one year of age.

The final conclusion of this thesis is that the prediction of dosing and drug response in children seems increasingly possible, be it through the use of allometric size models, physiologically-based developmental pharmacology models, or a combination of the two. As with the studies presented here on diclofenac, it is important for future pharmacokinetic and pharmacodynamic studies in children to also aim at improving the general understanding of developmental similarities and differences in drug handling between children and adults. Making accurate predictions of dosing and effect in paediatric subjects will enable optimisation of future clinical trials, help avoid unnecessary clinical trials being performed, and improve the speed and efficiency at which children gain to access new treatments, and benefit from existing therapies.

Appendix 7.1: Pharmacokinetic Study Ethical Approval

Institute of Child Health/Great Ormond Street Hospital Research Ethics Committee
The Institute of Child Health
30 Guilford Street
London
WC1N 1EH

Telephone: 020 7905 2620
Facsimile: 020 7905 2201

31 August 2005

Dr
Director of the Centre for Paediatric Pharmacy Research
Centre for Paediatric Pharmacy Research
London School of Pharmacy/Institute of Child Health
29/39 Brunswick Square
London
WC1N 1AX

Dear Dr Wong

Full title of study: **Population Pharmacokinetics of Diclofenac Suspension in Children Aged One to 12 Years**
REC reference number: **05/Q0508/83**

Thank you for your letter of 09 August 2005, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chairman considered at a Chair's action meeting held on 23 August 2005.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

The favourable opinion applies to the research sites listed on the attached form.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application		05 July 2005
Investigator CV	Dr Wong's CV	(None Specified)
Investigator CV	Dr Standing's CV	(None Specified)
Protocol	PKV001	27 June 2005

	27.06.2005	
Covering Letter		05 July 2005
GP/Consultant Information Sheets	GP letter, version 001	05 August 2005
Participant Information Sheet	Revised Parent Information Sheet, version 003a	05 August 2005
Participant Information Sheet	Revised 8 - 12 Information Sheet, version 002b	05 August 2005
Participant Information Sheet	Patient Information Sheet, under 8, version 001c	09 August 2005
Participant Information Sheet	Parent Information Sheet, version 002a	04 July 2005
Participant Information Sheet	Patient Information Sheet, version 001b	(None Specified)
Response to Request for Further Information		09 August 2005
Other	Clarification to LREC questions	(None Specified)
Other	MHRA algorith	(None Specified)

Management approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final management approval from the R&D Department for the relevant NHS care organisation.

Notification of other bodies

The Committee Administrator will notify the research sponsor and the R&D Department for NHS care organisation that the study has a favourable ethical opinion.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

SF1 list of approved sites

05/Q0508/83 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project,

Yours sincerely

Enclosures:

Standard approval conditions
Site approval form (SF1)

Appendix 7.2: Pharmacokinetic Study Parent Information Leaflet

Great Ormond Street Hospital for Children NHS Trust

To the Parent/Guardian of:

Diclofenac Pharmacokinetics in Children

This letter is to invite your child to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part. You can get more information about being involved in research through the patients and families section of the hospital website: *http://www.gosh.nhs.uk/gosh_families/research/index.html*

Thank you for reading this.

1. **What is the purpose of the study?**

Diclofenac is a medicine that is used for pain relief. This study looks at how diclofenac oral liquid is distributed in the body. This will increase our understanding of diclofenac in children so that we can give the most appropriate dose.

2. **Why has your child been chosen?**

Your child has been chosen because he/she is coming to hospital for an operation. They would normally receive diclofenac as a suppository or part of a soluble tablet for pain relief.

3. **Does your child have to take part?**

It is up to you to decide whether your child will take part. If you decide to take part you will be asked to sign a consent form. If you decide to take part you are free to withdraw your child from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

4. What will happen to your child if they take part?

If your child takes part, they will receive a dose of diclofenac oral liquid before the operation. When your child is asleep in the operating theatre, the anaesthetist will take two blood tests, one at the start of the operation and one at the end. A research nurse may also take blood tests after the operation, but only if you and your child give them permission. These blood tests will be taken from the cannula inserted into a vein for routine purposes, so there will be no extra needles involved. A test to look at a gene involved in the body's processing of diclofenac will be undertaken. This is to see whether individuals with a different gene type (polymorphism) process diclofenac in different ways. This testing will use a coded (anonymous) blood sample and we will not report any genetic information from which the individual concerned can be identified.

5. What is diclofenac?

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID). It is an effective pain reliever which can also help with swelling/inflammation if taken for longer periods.

6. What are the side-effects of diclofenac?

Like all medicines, there is a small chance that some people who take diclofenac will experience side-effects. The side-effects of diclofenac can be: digestive system upset (diarrhoea, sickness, tummy pain); changes in some blood tests such as decreased white cells or platelets, alteration in liver and kidney function blood tests; allergy (rash, wheeziness); cardiovascular changes (water retention, increased blood pressure).

Most of these are very rare and all should go away when the drug is stopped.

7. What are the disadvantages and benefits of taking part?

The disadvantage with taking part in this study is that your child will give some blood tests. These will be taken from the cannula (small tube inserted into a vein) which will be put there for the routine medications such as anaesthetics and antibiotics. Because we will take blood from the cannula which is inserted for other routine purposes, if your child takes part in the study, they will not have any extra procedure involving needles. There will be no direct benefit to your child, but taking part will help us to learn more about how diclofenac passes through their body. This may help those who receive it in the future.

8. **Will your child taking part in this study be kept confidential?**

All information which is collected about your child will be kept strictly confidential. Any information about your child which leaves the hospital will have the name and personal details removed so that they cannot be recognised from it. The research pharmacist, the pharmaceutical company sponsoring the project, and a representative of the research ethics committee will have access to the information about this project, but only the research pharmacist will be able to access the names of individual patients.

The use of some types of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. If you have any questions about data protection, contact the Data Protection officer via the switchboard on 020 7405 9200 extension 5217.

9. **What will happen to the results of the research study?**

We hope to publish the results of the study in a medical journal. Some of the information from this study may form part of an application for diclofenac to become licensed for use in children after surgery. No personal details of patients who took part will appear in any publication. Details of the study can be found by contacting the research pharmacist (details below).

10. **Who is organising and funding the research?**

This study is being organised by the Centre for Paediatric Pharmacy Research which is a part of the Great Ormond Street Hospital, Institute for Child Health (UCL) and the London School of Pharmacy. Funding is from Rosemont Pharmaceuticals. The Research Ethics Committee at Great Ormond Street Hospital has reviewed the study.

11 **What if something goes wrong?**

This project has been approved by an independent research ethics committee who believe that it is of minimal risk to your child. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. This has been arranged to meet standards set by a professional body, the Association of the British Pharmaceutical Industry, and a copy of their guidelines is available to you on request. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law.

This would require you to prove fault on the part of the Hospital/Institute and/or the manufacturer involved.

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the research pharmacist. If the problems are not resolved, or you wish to comment in any other way, please contact the sponsor of the study: Jeff Rothwell, Rosemont Pharmaceuticals, Yorkdale Industrial Estate, Braithwaite Street, Leeds, LS11 9XE, 01132977808.

Contact for Further Information

Joseph Standing, Research Pharmacist
Pharmacy Department
Great Ormond Street Hospital for Children
London
WC1N 3JH

Thank you for considering whether to take part in this study.

You should keep this information letter for future reference. If you decide to take part in the study you will also be given a copy of the signed consent form to keep.

Centre Number:
Study Number:
Patient Identification Number for this trial:

Appendix 7.3: Pharmacokinetic Study Patient Information Leaflets

Great Ormond Street Hospital for Children NHS Trust NHS

Diclofenac Study: Patient Information Sheet (8-12 years)

When you come to hospital for your operation, the doctor and pharmacist might decide to give you a medicine called diclofenac. Diclofenac is a medicine that stops you feeling pain. Our research project aims to help us find out more about how your body uses diclofenac. This will help us to work out the best dose to give.

Take time to decide if you want to say YES or NO to this. Please read, or have someone to read to you, this information. Don't worry if you don't understand it straight away. Your parents have also been told about this, and you can ask them to help you understand.

1) Why are we doing this?

We want to get more information on what happens when children like you take diclofenac. This will help us to understand the best dose to give so that children who take diclofenac at the time of an operation will have as little pain as possible.

2) What will be different for you?

Normally we would give you diclofenac as a soluble tablet before the operation or as a suppository whilst you are asleep in the operating theatre. If you decide to take part in the study, you will be given a dose of diclofenac liquid (medicine) before the operation. We will then take two blood tests in the operating theatre whilst you are asleep. If you give your permission, a research nurse may ask you for one or two other blood tests after the operation. These blood tests will be taken from the small tube (cannula) which is usually put into your hand during the operation.

3) Why do we ask you?

We ask you because you will soon be coming to hospital for an operation. When you do, you will be given diclofenac to decrease any pain.

4) Do I have to take part?

No. It is up to you and your parents to decide. If you decide you don't want to, that's absolutely fine. Everything that happens to you when you are in hospital will be just the same.

5/8/5 Version: 002b

5) What is diclofenac?

Diclofenac is a medicine that lowers pain. It can be given as a liquid (medicine), tablet, soluble tablet (tablet that can be mixed with water to make a drink), suppository (special tablet that goes into the bottom) or injection. If you take part in this study, you will be given diclofenac liquid (medicine) before the operation.

6) Is it dangerous?

We have given diclofenac to lots of children in the past. From this we think that side-effects are rare. Some people who take diclofenac get the following side-effects:

- Upset tummy
- Skin rashes
- Blood test problems
- Kidney or liver test problems

7) What about the results of the study?

What we find out about diclofenac will be written in a medical journal (magazine for people like doctors, pharmacists, nurses). Our study will show us more information on the best dose to give and will help the diclofenc medicine to be licensed. This will help children in the future to take diclofenac more easily and make sure they have the best possible pain relief.

8) Who will know that I am in the study?

The doctors, pharmacist and nurses taking care of you will know. So will the research pharmacist who is collecting the information.

We will not tell anyone else anything that will let them know who you are. When we write about diclofenac in the medical journal, we will not mention any details that will let people know who you are.

9) Who can I speak to if I have any questions?

You can speak to your parents who have also been given information about this project. You can also speak to the research pharmacist, Joe Standing, who will come and see you when you are in the hospital. If you or your parents have any other questions, your parents also have some further contact details for him. Also, all the doctors, pharmacists and nurses in the hospital can answer questions about diclofenac.

Great Ormond Street Hospital for Children NHS Trust

Patient Information Sheet (Under 8)

One of the medicines that you will be given when you come for your operation is called diclofenac. Diclofenac is a medicine that lowers pain. We are doing a study to look at how diclofenac liquid passes through your body.

Take time to decide if you want to say YES or NO to this. Please read this information, or ask someone to read it for you. Don't worry if you don't understand it straight away. Your parents have also been told about this, and you can ask them to help you understand.

This is a medicine called diclofenac, it lowers pain

The nurse will give you some before the operation

We want to know what happens to diclofenac when children take it

You can help. To help us we would like to take some blood tests

We can do this during the operation and maybe afterwards too

If you don't want to do this, it is OK. If you want to ask more, ask your Mum or Dad or Joe, the pharmacist

9/8/5 Version 001c

CASE REPORT FORM

Diclofenac Suspension Pharmacokinetics
Ethics Ref: 05/Q0508/83
R+D Ref: 05/VB/10

Patient ID: DPK_ _ _ _

Checklist:

1. All samples labeled/delivered ☐
2. Entry in notes/consent form included ☐
3. Follow-up phone call made ☐
4. Letter to GP ☐
5. All relevant CRF pages present/completed ☐

Signed: _____ Date: _____

CRF Cover Sheet V001 17.5.5.doc

Patient ID:

1. Patient

a. Age: __.__ b. Sex: M □1 F □2 c. Weight(kg): ___.__ d. Height (cm): ____

e. Ward: Cla □1 Hed □2 Lou □3 Rab □4 f. Surgeon:

Tig □5 PPan □6 Isl □7 DTC3 □8 g. Anaesthetist:

h. Procedure: Operation time:

i. Date of surgery (d/m/y) __/__/__ j. Date of discharge (d/m/y) __/__/__ k. Days in hosp: ___

l. Ethnic origin Asian □1 Black □2 Caucasian □3 Other □4

2. Past Medical History: **ASA Score:**

3. Drug History Known allergies/previous ADR's No□1 Yes□2

Details:

4. Recent Drug History: (inc. PoM, OTC, Herbal, Homeopathic medicines etc) None: □1

	Drug	Dose	Frequency	Taken in week prior to admission		Date stopped (d/m/y)	OR continues
1				Yes□1	No□2	__/__/__	□1
2				Yes□1	No□2	__/__/__	□1
3				Yes□1	No□2	__/__/__	□1
4				Yes□1	No□2	__/__/__	□1
5				Yes□1	No□2	__/__/__	□1
6				Yes□1	No□2	__/__/__	□1

4 (Cont.) Recent Drug History: (inc. PoM, OTC, Herbal, Homeopathic medicines etc)

	Drug	Dose	Frequency	Taken in week prior to admission		Date stopped (d/m/y)	OR continues
7				Yes☐1	No☐2	__/__/__	☐1
8				Yes☐1	No☐2	__/__/__	☐1
9				Yes☐1	No☐2	__/__/__	☐1
10				Yes☐1	No☐2	__/__/__	☐1
11				Yes☐1	No☐2	__/__/__	☐1
12				Yes☐1	No☐2	__/__/__	☐1
13				Yes☐1	No☐2	__/__/__	☐1
14				Yes☐1	No☐2	__/__/__	☐1
15				Yes☐1	No☐2	__/__/__	☐1
16				Yes☐1	No☐2	__/__/__	☐1
17				Yes☐1	No☐2	__/__/__	☐1
18				Yes☐1	No☐2	__/__/__	☐1

5. Diclofenac Dosing

	Drug	Dose	Frequency				Form		Date (d/m/y) of first dose	Comments
	Diclofenac		Day	1	2	3	Suspension:			
			1				Suppository:			
			2				Sol. tab.:			
			3				e/c tab:			
			4				I/M:			
							I.V:			

6. Blood Sampling

	Timing				Sample identification number	Comments
	Day	1	2	3		
	1				DPK	
	2				DPK	
	3				DPK	
	4				DPK	
					DPK	
					DPK	

Patient ID:

7. Inpatient Medications: (Week: _____)

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)	OR continued
1			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
2			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
3			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
4			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
5			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
6			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1

Patient ID:

7. Inpatient Medications: (Week: _____)

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)	OR continued
7			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
8			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
9			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
10			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
11			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
12			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1

8. Discharge Medications: None ☐1

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)
1				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
2				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
3				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
4				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
5				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
6				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1

8a. Discharge Medications: None ☐1

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)
7				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
8				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
9				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
10				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
11				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
12				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1

9. Laboratory Abnormalities (outside ref. range): Blood tests performed: Yes ☐1 No ☐2 b. Abnormal Results Yes ☐1 No ☐2

Parameter (Range)	Pre-operative __/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__

Patient ID:

10. Adverse Events: (Week:) None: ☐1 (add any additional relevant information on 10b.)

	Identify event/any treatment given	Time of onset (O) / resolution (R)	Reported by	Event Resolved	Serious*	OR	Infection
1		Day 1 2 3 4 5 6 7 Time	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐2 ☐3 ☐4 ☐5 ☐6		Yes ☐1 No ☐2
2		Day 1 2 3 4 5 6 7 Time	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐2 ☐3 ☐4 ☐5 ☐6		Yes ☐1 No ☐2
3		Day 1 2 3 4 5 6 7 Time	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐2 ☐3 ☐4 ☐5 ☐6		Yes ☐1 No ☐2
4		Day 1 2 3 4 5 6 7 Time	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐2 ☐3 ☐4 ☐5 ☐6		Yes ☐1 No ☐2
5		Day 1 2 3 4 5 6 7 Time	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐2 ☐3 ☐4 ☐5 ☐6		Yes ☐1 No ☐2

*Seriousness:

1. Fatal
2. Life-threatening
3. Prolonged hospitalisation
4. Persistent or significant disability/incapacity
5. Other serious event requiring medical or surgical intervention to prevent any of the above (1-4)
6. Not serious.

Patient ID:

10a. Adverse Events: (Week:) None: ☐1 (add any additional relevant information on 10b.)

	Identify event/any treatment given	Time of onset (O) / resolution (R)	Reported by	Event Resolved	Serious*	OR	Infection
6		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
7		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
8		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
9		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
10		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2 ? ☐3	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2

*Seriousness:

1. Fatal
2. Life-threatening
3. Prolonged hospitalisation
4. Persistent or significant disability/incapacity
5. Other serious event requiring medical or surgical intervention to prevent any of the above (1-4)
6. Not serious.

Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – DAY 1 POST OP.

Have you/your child had any problems since the operation? Yes□$_1$ No□$_2$
□ Clarify DH if necessary □ Establish time of onset

Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – TELEPHONE INTERVIEW

Have you/your child had any problems since leaving hospital? Yes□$_1$ No□$_2$

- □ Establish time of onset/resolution
- □ When was diclofenac stopped/number of doses?

11. Research Pharmacist Causality Assessment			
AE no.	Naranjo *et al* Algorithm	Jones *et al* Algorithm	WHO Criteria
1	Score ____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
2	Score ____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
3	Score ____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
4	Score ____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
5	Score ____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6

Appendix 7.5: NONMEM Control File for Final Pharmacokinetic Model

```
$PROBLEM Two Absorption Comp Model, POOLED DATA;

$DATA c:\PKdatafiles\MOLARPOOLED.csv IGNORE=@

$INPUT ID OCC TIME AMT DV MDV EVID WT HT AGE SEX RACE FORM CMT STDY CYP

$SUBROUTINES ADVAN6 TOL=5

$MODEL

 COMP = (ABS1)
 COMP = (ABS2)
 COMP = (CENTRAL)

$PK
 IF(OCC.EQ.1)THEN     ; Between occasion variability
 BOVV  = ETA(10)
 BOVCL = ETA(11)
 ENDIF

 IF(OCC.EQ.2)THEN
 BOVV  = ETA(12)
 BOVCL = ETA(13)
 ENDIF

 IF(AMT.GT.0.AND.CMT.EQ.1)PODO1=AMT ; COMP1 Oral dosing
 IF(AMT.GT.0.AND.CMT.EQ.2)PODO2=AMT ; COMP2 Oral dosing

 F1   = 0
 F2   = 0
 F3   = 1

 TVBIO1 = THETA(1)
 AB     = 1 - TVBIO1
 AA     = LOG(TVBIO1/AB)
 BIO1   = EXP(AA+ETA(1))/(1 + EXP(AA + ETA(1)))
 BIO2   = 1 - BIO1

 TA1    = THETA(2)*EXP(ETA(2))
 MTT1   = THETA(3)*EXP(ETA(3))              ; COMP1 MEAN TRANSIT TIME
 N1     = THETA(4)*EXP(ETA(4))              ; COMP1 NO TRANSIT COMPS
 KTR1   = (N1+1)/MTT1                       ; COMP1 TRANSIT RATE CONSTANT

 LNFAC1 = LOG(2.5066) + (N1+0.5)*LOG(N1)-N1   ; COMP1 LN STERLING APPROXIMATION

 TA2    = THETA(5)*EXP(ETA(5))
 MTT2   = THETA(6)*EXP(ETA(6))              ; COMP2 MEAN TRANSIT TIME
 N2     = THETA(7)*EXP(ETA(7))              ; COMP2 NO TRANSIT COMPS
 KTR2   = (N2+1)/MTT2                       ; COMP2 TRANSIT RATE CONSTANT

 LNFAC2 = LOG(2.5066) + (N2+0.5)*LOG(N2)-N2   ; COMP2 LN STERLING APPROXIMATION

 TVV3 = THETA(8)
 V3   = (TVV3*(WT/70))*EXP(ETA(8)+BOVV)
 TVCL = THETA(9)
 CL   = (TVCL*((WT/70)**0.75))*EXP(ETA(9)+BOVCL)

 LN2  = LOG(2)
 K13  = LN2/TA1
 K23  = LN2/TA2
 KE   = CL/V3
 S3   = V3
```

```
$DES

 DADT(1) = EXP(LOG(BIO1*PODO1+0.00001)+LOG(KTR1)+N1*LOG(KTR1*T+0.00001)-KTR1*T-LNFAC1) -
K13*A(1)
 DADT(2) = EXP(LOG(BIO2*PODO2+0.00001)+LOG(KTR2)+N2*LOG(KTR2*T+0.00001)-KTR2*T-LNFAC2) -
K23*A(2)
 DADT(3) = K13*A(1) + K23*A(2) - KE*A(3)

$ERROR (ONLY OBSERVATIONS)    ; Residual variability

 IPRED = A(3)/V3
 IRES  = DV-IPRED
 IF(STDY.EQ.0)THEN            ; Study=0 = Adult study
 W     = THETA(10)*IPRED
 ENDIF
 IF(STDY.EQ.1)THEN            ; Study=1 = Paediatric study
 W     = THETA(11)*IPRED
 ENDIF
 IF(W.EQ.0) W = 1
 IWRES = IRES/W

 Y = IPRED + W*EPS(1)

$THETA (0,0.7,1)    ; 1 BIO1
$THETA (0,0.1,1)    ; 2 TA1
$THETA (0,0.7,3)    ; 3 MTT1
$THETA (0,1,5)      ; 4 N1
$THETA (0,1,2)      ; 5 TA2
$THETA (0,1.3,3)    ; 6 MTT2
$THETA (0,41,100)   ; 7 N2
$THETA (0,5,50)     ; 8 V3
$THETA (0,50,100)   ; 9 CL
$THETA (0,0.21)     ; 10 Prop err adult
$THETA (0,0.18)     ; 11 Prop err child

$OMEGA 1            ; 1 BIO1
$OMEGA 1.8          ; 2 TA1
$OMEGA 0.02         ; 3 MTT1
$OMEGA 0.6          ; 4 N1
$OMEGA 0.2          ; 5 TA2
$OMEGA 0.02         ; 6 MTT2
$OMEGA 2            ; 7 N2
$OMEGA BLOCK(2)
0.3
0.003  0.06         ; 8 V3 9 CL
$OMEGA BLOCK(2)
0.9
0.002 0.04          ; 10 BOVV 11 BOVCL
$OMEGA BLOCK(2) SAME
;
;                   ; 12 BOVV 13 BOVCL

$SIGMA 1 FIX

$ESTIMATION PRINT=10 METHOD=1 INTER SIGDIG=4 NOABORT MAXEVAL=9999 POSTHOC MSFO=msfb200

$COVARIANCE MATRIX=S

$TABLE ID TIME IPRED IWRES IRES          NOPRINT ONEHEADER FILE=sdtab200
$TABLE ID CL V3 AGE WT HT SEX CYP        NOPRINT ONEHEADER FILE=patab200
$TABLE ID AGE WT HT                      NOPRINT ONEHEADER FILE=cotab200
$TABLE ID SEX RACE CYP                   NOPRINT ONEHEADER FILE=catab200
```

Appendix 7.6: Safety Study Ethical Approval

Institute of Child Health/Great
Ormond Street Hospital Research
Ethics Committee
The Institute of Child Health
30 Guilford Street
London
WC1N 1EH

16 April 2004

Dr Ian Wong
Director of the Centre for Paediatric
Pharmacy Research
Centre for Paediatric Pharmacy Research
London School of Pharmacy
29-39 Brunswick Square
London
WC1N 1AX

Dear Dr Wong

Full title of study: **The Safety of Diclofenac for Post-Operative Pain Relief in Children DISCOS (Diclofenac Safety in Children for post-operative pain: Observational Study)**
REC reference number: **04/Q0508/3**
Protocol number: **03**
GOSH/ICH R&D Number: **04VB02**

The Research Ethics Committee reviewed the above application at the meeting held on 07 April 2004. Many thanks to Mr Standing for attending the meeting.

Ethical opinion

The committee noted that the researchers have discussed the study with the departments who will be involved before the submission to the REC was made.

The committee felt that the researchers had made a very thoughtful application with regards to the study design, approach, etc.

The information sheet was considered good.

The committee asked Mr Standing how he would identify whether any patients were already involved in another trial. Mr Standing explained that he will address this issue with the doctors looking after the patients. The committee noted that participation in one other trial should not necessarily exclude families from taking part in this project.

The committee also asked Mr Standing how it will be determined that any adverse events are due to diclofenac, when patients will be taking other drugs. Mr Standing explained that a causality assessment will be performed in order to reach a decision between the team about how likely an event is to be due to diclofenac. There is some literature for criteria for this assessment. As diclofenac is used as a more potent therapy, it would not really be possible to carry out the research in patients taking less drugs, although some 'less complicated' patients will be looked at when the study is extended to Guys.

The members of the Committee present gave a favourable ethical opinion to the above research on the basis described in the application form, protocol and supporting documentation.

The favourable opinion applies to the research sites listed on the attached sheet. Confirmation of approval for other sites listed in the application will be issued as soon as local assessors have confirmed that they have no objection.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The documents reviewed and approved at the meeting were:

Document Type: Application

Document Type: Investigator CV

Document Type: Protocol
Version: 03
Dated: 16/03/2004

Document Type: Participant Information Sheet
Dated: 09/03/2004

Management approval

If you are the Principal Investigator for the lead site: You should obtain final management approval from your host organisation before commencing this research.

The study should not commence at any other site until the local Principal Investigator has obtained final management approval from the relevant host organisation.

All researchers and research collaborators who will be participating in the research must obtain management approval from the relevant host organisation before commencing any research procedures. Where a substantive contract is not held with the host organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Yours sincerely,

Enclosures *List of names and professions of members who were present at the meeting and those who submitted written comments; Standard approval conditions; List of approved sites*

Copy to: *R&D Office, ICH; Mr J Standing*

Appendix 7.7: Safety Study Parent Information Leaflet

Great Ormond Street Hospital for Children NHS Trust

To the Parent/Guardian of:

The Safety of Diclofenac for Post-Operative Pain Relief in Children

This letter is to invite your child to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please contact us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part.

Thank you for reading this.

1 What is the purpose of the study?

Diclofenac is a medicine that is used for pain relief. Although it is regularly prescribed for children, diclofenac is only available as tablets, soluble tablets or suppositories. Our study aims to increase the understanding of diclofenac in children to provide information for the licensing of a children's liquid.

2. Why has your child been chosen?

Your child has been chosen because he/she might come to hospital for an operation and so it is quite likely they will receive diclofenac for pain relief.

3. Does your child have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be asked to sign a consent form in the pre-admission clinic. If you decide to take part you are free to withdraw your child from the study at any time without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care your child receives.

4. What will happen to your child if they take part?

The doctors and pharmacists taking care of your child will decide whether or not to prescribe diclofenac, this will not be affected by taking part in this project. The research pharmacist will come and see you on the day after the operation or phone you if you've gone home. If your child has been prescribed diclofenac he/she will be enrolled in the study. The research pharmacist will come back to see you two days later and every week whilst you are still in hospital. He will

also telephone you one week after discharge. You will be asked about any problems your child may have experienced after the operation. Your child's medical records for the days when he/she is in hospital will be looked at and the results of any blood tests will be recorded. There will be no extra blood tests and nothing extra will be physically done as a result of participating in this study.

5. What if your child is not prescribed diclofenac?

The decision to prescribe diclofenac will be made by the doctors and pharmacists looking after your child. If they decide not to prescribe diclofenac we may still ask permission to collect some information about your child to see if there are differences between those who are and are not prescribed diclofenac. This will only happen at certain times in the study and the research pharmacist will let you know if any information about your child will be collected.

6. What is diclofenac?

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID). It is licensed for adults for many conditions including pain relief after an operation and has been available for well over 20 years. As with many drugs, because not enough studies have been done involving children, diclofenac does not have a license for use in children. Although diclofenac has been found to be effective and is widely used in children, more information about its safety is needed before it can be licensed for children. Currently, diclofenac is only available as tablets, soluble tablets, suppositories and injection. This study may help diclofenac to become licensed for children and allow the development of a liquid form which children might find easier to take.

7. What are the side-effects of diclofenac?

Like all medicines, there is a chance that some people who take diclofenac will experience side-effects. The main side-effects of diclofenac when used in adults are:

- Digestive system upset: diarrhoea, sickness, tummy pain.
- Changes in some blood tests: decreased white cells or platelets, alteration in liver and kidney function blood tests.
- Allergy: rash, wheeziness.
- Cardiovascular changes: water retention, increased blood pressure.

Most of these are very rare and go away when the drug is stopped. This study aims to see if similar side-effects happen in children and how common they are.

8. **What are the disadvantages and benefits of taking part?**

There are no disadvantages with taking part in this study but there will be no direct benefit to your child. Every part of the care your child receives will be the same whether or not he/she takes part. By taking part you will help us to learn more about what effects diclofenac has on children. This may help those who receive it in the future.

9. **Will your child taking part in this study be kept confidential?**

All information which is collected about your child will be kept strictly confidential. Any information about your child which leaves the hospital will have the name and personal details removed so that they cannot be recognised from it. The research pharmacist, the pharmaceutical company sponsoring the project, and a representative of the research ethics committee will have access to the information about this project.

The use of some types of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to people about whom information is held. If you have any questions about data protection, contact the Data Protection officer via the switchboard on 020 7405 9200 extension 5217.

10. **What will happen to the results of the research study?**

We hope to publish the results of the study in a medical journal. Some of the information from this study may form part of an application for diclofenac to become licensed for use in children after surgery. No personal details of patients who took part will appear in any publication. Details of the study can be found by contacting the research pharmacist (details below).

11. **Who is organising and funding the research?**

This study is being organised by the Centre for Paediatric Pharmacy Research which is a part of the Great Ormond Street Hospital, Institute for Child Health (UCL) and the London School of Pharmacy. Funding is from Rosemont Pharmaceuticals who hope to use the results to support an application for diclofenac to become licensed. The Research Ethics Committee at Great Ormond Street Hospital has reviewed the study.

12. **What if something goes wrong?**

This project has been approved by an independent research ethics committee who believe that it is of minimal risk to your child. However, research can carry unforeseen risks and we want you to be informed of your rights in the unlikely

event that any harm should occur as a result of taking part in this study.

This research is covered by a no-fault compensation scheme which may apply in the event of any significant harm resulting to your child from involvement in the study. This has been arranged to meet standards set by a professional body, the Association of the British Pharmaceutical Industry, and a copy of their guidelines is available to you on request. Under this scheme it would not be necessary for you to prove fault. You also have the right to claim damages in a court of law. This would require you to prove fault on the part of the Hospital/Institute and/or the manufacturer involved.

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the research pharmacist. If the problems are not resolved, or you wish to comment in any other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guildford Street, London WC1N 3EH or if urgent, by telephone on 0207 905 2620 and the Committee administration will put you in contact with him.

Contact for Further Information

Joseph Standing, Research Pharmacist
Pharmacy Department
Great Ormond Street Hospital for Children
London
WC1N 3JH

Thank you for considering whether to take part in this study.

You should keep this information letter and bring it when you next come to hospital. If you decide to take part in the study you will also be given a copy of the signed consent form to keep.

Centre Number:
Study Number:
Patient Identification Number for this trial:

Appendix 7.8: Safety Study Patient Information Leaflet

Great Ormond Street Hospital for Children NHS Trust

Diclofenac Study: Patient Information Sheet

When you come to hospital for your operation, the doctor and pharmacist might decide to give you a medicine called diclofenac. Diclofenac is a medicine that stops you feeling pain. Our research project aims to help us find out more about how diclofenac works in children like yourself.

Take time to decide if you want to say YES or NO to this. Please read, or have someone to read to you, this information. Don't worry if you don't understand it straight away. Your parents have also been told about this, and you can ask them to help you understand.

1) Why are we doing this?

Nobody has done a project that looks at the safety of diclofenac for children. With all medicines there is a small chance of side-effects. A side-effect is something caused by the medicine that is unwanted. For example, some medicines for hay fever can also make you sleepy; this is a side-effect. We want to see if diclofenac causes side-effects when children take it.

2) What will be different for you?

Nothing will be different for you if you decide to take part. The doctor and pharmacist on the ward will decide if you need diclofenac during or after your operation. The research pharmacist, Joe Standing, will come and see you on the day after your operation. Joe will tell you if you have had diclofenac. If you have, he will ask you about any problems you have had. Joe will write down everything you tell him. He will also look at the notes that the doctors and nurses on the ward write about how you are. Joe will come and see you every few days while you are in hospital and phone your parents one week after you go home

3) Why do we ask you?

We ask you because you might come to hospital for an operation. If you do, you may be given diclofenac when you are in hospital.

4) Do I have to take part?

No. It is up to you and your parents to decide. If you decide you don't want to, that's absolutely fine. Everything that happens to you when you are in hospital will be just the same.

9/3/04 Version: 002d

5) What is diclofenac?

Diclofenac is a medicine that lowers pain. It can be given as a tablet, soluble tablet (tablet that can be mixed with water to make a drink), suppository (special tablet that goes into the bottom) or injection.

6) Is it dangerous?

We have given diclofenac to lots of children in the past. From this we think that side-effects are quite rare. When grown-ups take diclofenac they sometimes get the following side-effects:

- Upset tummy
- Skin rashes
- Blood test problems
- Kidney or liver problems

These side-effects are rare in grown-ups. We want to check whether they are also rare in children.

7) What about the results of the study?

What we find out about diclofenac will be written in a medical journal (magazine for people like doctors, pharmacists, nurses). If we find out that side-effects are rare for children, it might mean that a special children's liquid diclofenac can be made. This will help children in the future to take diclofenac more easily.

8) Who will know that I am in the study?

The doctors, pharmacist and nurses taking care of you will know. So will the research pharmacist who is collecting the information.

We will not tell anyone else anything that will let them know who you are. When we write about diclofenac in the medical journal, we will not mention any details that will let people know who you are.

9) Who can I speak to if I have any questions?

You can speak to your parents who have also been given information about this project. You can also speak to the research pharmacist, Joe Standing, who will come and see you when you are next in the hospital. If you or your parents have any other questions, your parents also have some further contact details for him. Also, the doctors, pharmacists and nurses in the hospital can answer questions about diclofenac.

9/3/04 Version: 002d

CASE REPORT FORM

Diclofenac adverse events monitoring in post-operative paediatric patients
Acronym: DIclofenac Safety in Children: use in post-Operative pain (DISCO)

Patient ID: _ _ _ _

Checklist:

1. Recruited from:	Pre-admission clinic ☐1	Postal consent ☐2
2. Consent form signed and copies made	☐	
3. Date of admission recorded	☐	
4. Received diclofenac	Yes ☐1	No ☐2
5. Entry in notes/consent form included	☐	
6. CRF completed	☐	

Patient ID:

1. Patient

a. Age: __.__ b. Sex: M □1 F □2 c. Weight(kg): __.__

d. Ward: Cla □1 Hed □2 Lou □3 Rab □4 e. Surgeon:

Tig □5 PPan □6 Isl □7 DTC3 □8 f. Anaesthetist:

g. Procedure: _____

h. Date of surgery (d/m/y) __/__/__ i. Date of discharge (d/m/y) __/__/__ j. Days in hosp:

k. Ethnic origin Asian □1 Black □2 Caucasian □3 Other □4

2. Past Medical History:

_____

_____

_____

_____

3. Drug History **Known allergies/previous ADR's** **No□1 Yes□2**

Details: _____

_____

4. Recent Drug History: (inc. PoM, OTC, Herbal, Homeopathic medicines etc) None: □1

	Drug	Dose	Frequency	Taken in week prior to admission		Date stopped (d/m/y)	OR continues
1				Yes□1	No□2	__/__/__	□1
2				Yes□1	No□2	__/__/__	□1
3				Yes□1	No□2	__/__/__	□1
4				Yes□1	No□2	__/__/__	□1
5				Yes□1	No□2	__/__/__	□1
6				Yes□1	No□2	__/__/__	□1

Patient ID:

5. Inpatient Medications: (Week: _____)

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)	OR continued
1	Diclofenac: Sol. Tab. ☐1 E/c Tablet ☐2 Liquid ☐3 Suppository ☐4 Injection ☐5		Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
2			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
3			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
4			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
5			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1
6			Day 1 2 3 4 5 6 7 Time	PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__	☐1

Patient ID:

5. Inpatient Medications: (Week:)

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)	OR continued
7			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1
8			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1
9			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1
10			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1
11			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1
12			Day 1 2 3 4 5 6 7 Time	PO □1 PR □2 IV □3 SC □4 IM □5 Other: ____	__/__/__	__/__/__	□1

Patient ID:_

6. Discharge Medications: None ☐1

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)
1				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
2				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
3				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
4				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
5				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1
6				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__ OR prior to admission ☐1	__/__/__ OR continued ☐1

Patient ID:

6 a. Discharge Medications Continued:

	Drug	Dose	Frequency	Route	Date started (d/m/y)	Date stopped (d/m/y)
7				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1
8				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1
9				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1
10				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1
11				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1
12				PO ☐1 PR ☐2 IV ☐3 SC ☐4 IM ☐5 Other: ____	__/__/__	__/__/__
					OR prior to admission ☐1	OR continued ☐1

7. Laboratory Abnormalities (outside reference range):

a. Blood tests performed: Yes ☐1 No ☐2 b. Abnormal Results Yes ☐1 No ☐2

Parameter (Range)	Pre-operative __/__/__	__/__/__	__/__/__	__/__/__	__/__/__	__/__/__

Patient ID:

8. Adverse Events: (Week:) None: ☐1 (add any additional relevant information on 8a.)

	Identify event/any treatment given	Time of onset (O) / resolution (R)	Reported by	Event Resolved	Serious*	OR	Infection
1		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
2		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
3		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
4		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
5		Day 1 2 3 4 5 6 7 T i m e	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2

*Seriousness:

1. Fatal
2. Life-threatening
3. Prolonged hospitalisation
4. Persistent or significant disability/incapacity
5. Other serious event requiring medical or surgical intervention to prevent any of the above (1-4)
6. Not serious.

8 a. Adverse events (additional information):	

Patient ID:

8 b. Adverse Events: (Week: _____) (add any additional relevant information on 8a.)

	Identify event/any treatment given	Time of onset (O) / resolution (R)	Reported by	Event Resolved	Serious*	OR	Infection
6		Day 1 2 3 4 5 6 7 Time O R	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
7		Day 1 2 3 4 5 6 7 Time O R	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
8		Day 1 2 3 4 5 6 7 Time O R	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
9		Day 1 2 3 4 5 6 7 Time O R	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2
10		Day 1 2 3 4 5 6 7 Time O R	Med. notes ☐1 Nurse notes ☐2 Patient ☐3 Parent ☐4 Path. Lab. ☐5 Ward Staff ☐6	Yes ☐1 No ☐2	☐1 ☐4 ☐6 ☐2 ☐5 ☐3		Yes ☐1 No ☐2

*Seriousness:

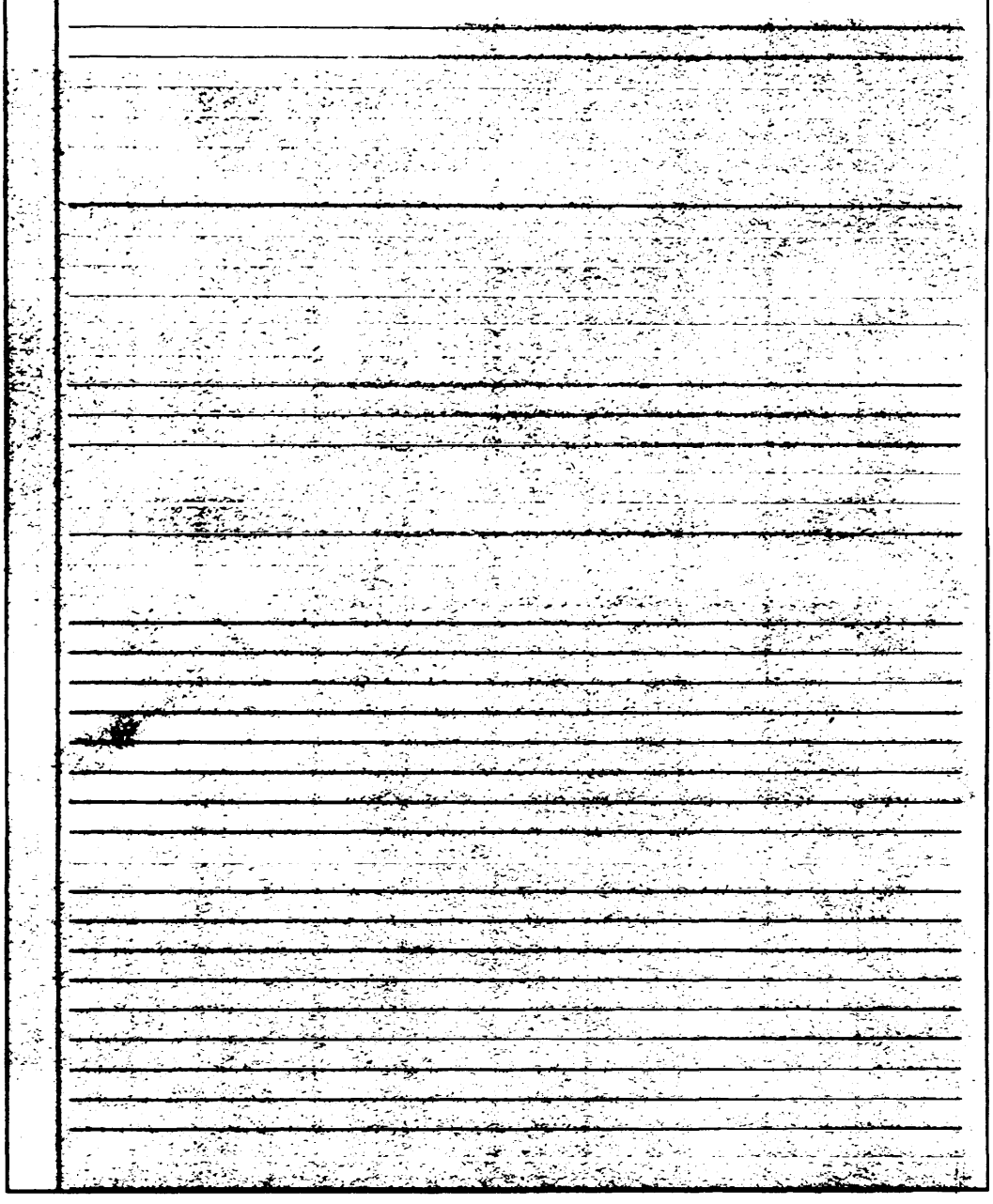
1. Fatal
2. Life-threatening
3. Prolonged hospitalisation
4. Persistent or significant disability/incapacity
5. Other serious event requiring medical or surgical intervention to prevent any of the above (1-4)
6. Not serious.

Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – DAY 1 POST OP.

Have you/your child had any problems since the operation? Yes□$_1$ No□$_2$

- □ Clarify DH if necessary
- □ Establish time of onset



Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – DAY 3 POST OP.

Have you/your child had any problems since the operation? Yes□$_1$ No□$_2$	
	□ Establish time of onset/resolution

Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – DAY 3 POST OP.

Have you/your child had any problems since the operation? Yes□$_1$ No□$_2$	
	□ Establish time of onset/resolution

Patient ID:____

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – WEEK

Have you/your child had any problems since the operation? Yes□$_1$ No□$_2$
□ Establish time of onset/resolution

Patient ID:

RECORD OF OPEN QUESTIONS TO PATIENT/PARENT – TELEPHONE INTERVIEW

Have you/your child had any problems since leaving hospital? Yes□$_1$ No□$_2$

- □ Establish time of onset/resolution
- □ When was diclofenac stopped/number of doses?

9. Research Pharmacist Causality Assessment			
AE no.	Naranjo *et al* Algorithm	Jones *et al* Algorithm	WHO Criteria
1	Score _____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
2	Score _____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
3	Score _____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
4	Score _____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6
5	Score _____ Definite: ☐1 Probable ☐2 Possible: ☐3 Unlikely: ☐4	Highly Probable: ☐1 Probable: ☐2 Possible: ☐3 Remote: ☐4	Very likely/Certain: ☐1 Probable/likely: ☐2 Possible: ☐3 Unlikely: ☐4 Unrelated: ☐5 Unclassifiable: ☐6

Appendix 7.10: Reference List for Studies Included for the Final Screen of the Systematic Literature Review

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Appendix 7.11: NONMEM Control File for Michaelis-Menten 4'-Hydroxydiclofenac Appearance Model

```
$PROBLEM Two Comp Two Abs Comp Metab Model MMAPP;

$DATA c:\PKdatafiles\MOLARPOOLED4OH.csv IGNORE=@

$INPUT ID OCC TIME AMT DV MDV EVID WT HT AGE SEX RACE FORM CMT STDY CYP

$SUBROUTINES ADVAN6 TOL=5

$MODEL

 COMP = (ABS1)
 COMP = (ABS2)
 COMP = (CENTRAL)
 COMP = (4OH)

$PK

 IF(OCC.EQ.1)THEN
 BOVV   = ETA(13)
 BOVCLD = ETA(14)
 ENDIF

 IF(OCC.EQ.2)THEN
 BOVV   = ETA(15)
 BOVCLD = ETA(16)
 ENDIF

 IF(AMT.GT.0.AND.CMT.EQ.1)PODO1=AMT ; COMP1 Oral dosing
 IF(AMT.GT.0.AND.CMT.EQ.2)PODO2=AMT ; COMP2 Oral dosing

 F1   = 0
 F2   = 0
 F3   = 1

 TVBIO1 = THETA(1)
 AB     = 1 - TVBIO1
 AA     = LOG(TVBIO1/AB)
 BIO1   = EXP(AA+ETA(1))/(1 + EXP(AA + ETA(1)))
 BIO2   = 1 - BIO1

 TA1    = THETA(2)*EXP(ETA(2))
 MTT1   = THETA(3)*EXP(ETA(3))                ; COMP1 MEAN TRANSIT TIME
 N1     = THETA(4)*EXP(ETA(4))                ; COMP1 NO TRANSIT COMPS
 KTR1   = (N1+1)/MTT1                         ; COMP1 TRANSIT RATE CONSTANT

 LNFAC1 = LOG(2.5066) + (N1+0.5)*LOG(N1)-N1   ; COMP1 LN STERLING APPROXIMATION

 TA2    = THETA(5)*EXP(ETA(5))
 MTT2   = THETA(6)*EXP(ETA(6))                ; COMP2 MEAN TRANSIT TIME
 N2     = THETA(7)*EXP(ETA(7))                ; COMP2 NO TRANSIT COMPS
 KTR2   = (N2+1)/MTT2                         ; COMP2 TRANSIT RATE CONSTANT

 LNFAC2 = LOG(2.5066) + (N2+0.5)*LOG(N2)-N2   ; COMP2 LN STERLING APPROXIMATION


 V3   = (THETA(8)*(WT/70))*EXP(ETA(8)+BOVV)
 CLD  = (THETA(9)*((WT/70)**0.75))*EXP(ETA(9)+BOVCLD)

 VM    = THETA(10)*EXP(ETA(10))
 KM    = THETA(11)*EXP(ETA(11))

 CLI   = (VM/KM)*((WT/70)**0.75)
 CL4H  = (THETA(12)*((WT/70)**0.75))*EXP(ETA(12))
```

```
 LN2   = LOG(2)
 K13   = LN2/TA1
 K23   = LN2/TA2
 KE    = CLD/V3
 V4    = 30*(WT/70)
 KE4   = CL4H/V4
 S3    = V3
 S4    = V4

$DES
 CD = A(3)/V3

 DADT(1) = EXP(LOG(BIO1*PODO1+0.00001)+LOG(KTR1)+N1*LOG(KTR1*T+0.00001)-KTR1*T-LNFAC1) -
K13*A(1)
 DADT(2) = EXP(LOG(BIO2*PODO2+0.00001)+LOG(KTR2)+N2*LOG(KTR2*T+0.00001)-KTR2*T-LNFAC2) -
K23*A(2)
 DADT(3) = K13*A(1) + K23*A(2) - KE*A(3) - CD*VM/(KM+CD)
 DADT(4) = (CD*VM/(KM+CD)) - KE4*A(4)


$ERROR (ONLY OBSERVATIONS)

 IPDICL = A(3)/V3
 IPD4OH = A(4)/(30*(WT/70))

 IF(CMT.EQ.3)THEN
 IPRED = IPDICL
 ENDIF

 IF(CMT.EQ.4)THEN
 IPRED = IPD4OH
 ENDIF

 IRES  = DV-IPRED
 IF(STDY.EQ.0)THEN
 W3     = THETA(13)*IPRED
 ENDIF
 IF(STDY.EQ.1)THEN
 W3     = THETA(14)*IPRED
 W4     = THETA(15)*IPRED
 ENDIF

 IF(CMT.EQ.3)THEN
 W = W3
 ENDIF

 IF(CMT.EQ.4)THEN
 W = W4
 ENDIF

 IF(W.EQ.0) W = 1

 IWRES = IRES/W

 Y = IPRED + W*EPS(1)

$THETA (0,0.7,1)    ; 1 BIO1
$THETA (0,0.1,1)    ; 2 TA1
$THETA (0,0.7,3)    ; 3 MTT1
$THETA (0,1,5)      ; 4 N1
$THETA (0,1,2)      ; 5 TA2
$THETA (0,1.3,3)    ; 6 MTT2
$THETA (0,41,100)   ; 7 N2
$THETA (0,5,50)     ; 8 V3
$THETA (0,48,100)   ; 9 CLD
$THETA (0,81700)        ; 10 VM
$THETA (0,13200)        ; 11 KM
$THETA (0,15,200)       ; 12 CL4H
$THETA (0,0.28)         ; 13 Prop err adult
$THETA (0,0.18)         ; 14 Prop err child diclo
$THETA (0,0.3)          ; 15 Prop err child 4OH
```

```
$OMEGA 1            ; 1 BIO1
$OMEGA 1.8          ; 2 TA1
$OMEGA 0.02         ; 3 MTT1
$OMEGA 0.6          ; 4 N1
$OMEGA 0.2          ; 5 TA2
$OMEGA 0.02         ; 6 MTT2
$OMEGA 2            ; 7 N2
$OMEGA BLOCK(2)
0.3
0.003  0.06         ; 8 V3 9 CL
$OMEGA 0.07         ; 10 VM
$OMEGA 0.04         ; 11 KM
$OMEGA 0.03         ; 12 CL4H
$OMEGA BLOCK(2)
0.7
0.002 0.08          ; 13 BOVV 14 BOVCL
$OMEGA BLOCK(2) SAME
;
;                   ; 15 BOVV 16 BOVCL

$SIGMA 1 FIX

$ESTIMATION PRINT=10 METHOD=1 INTER SIGDIG=5 NOABORT MAXEVAL=9999 POSTHOC MSFO=msfb301

$COVARIANCE MATRIX=S

$TABLE ID TIME IPRED IWRES IRES                          NOPRINT ONEHEADER FILE=sdtab301
$TABLE ID AGE WT HT CYP                                  NOPRINT ONEHEADER FILE=cotab301
$TABLE ID AGE SEX RACE CYP WT                            NOPRINT ONEHEADER FILE=catab301
$TABLE ID CMT TIME AGE WT CYP VM KM CL4H IPRED CLI       NOPRINT ONEHEADER FILE=patab301
```

Appendix 7.12: NONMEM Control File for First-Order 4'-Hydroxydiclofenac Appearance Model

```
$PROBLEM Two Comp Two Abs Comp Metab Model FOAPP;

$DATA c:\PKdatafiles\MOLARPOOLED4OH.csv IGNORE=@

$INPUT ID OCC TIME AMT DV MDV EVID WT HT AGE SEX RACE FORM CMT STDY CYP

$SUBROUTINES ADVAN6 TOL=5

$MODEL

 COMP = (ABS1)
 COMP = (ABS2)
 COMP = (CENTRAL)
 COMP = (4OH)

$PK
 IF(OCC.EQ.1)THEN
 BOVV   = ETA(13)
 BOVCLD = ETA(14)
 ENDIF

 IF(OCC.EQ.2)THEN
 BOVV   = ETA(15)
 BOVCLD = ETA(16)
 ENDIF

 IF(AMT.GT.0.AND.CMT.EQ.1)PODO1=AMT ; COMP1 Oral dosing
 IF(AMT.GT.0.AND.CMT.EQ.2)PODO2=AMT ; COMP2 Oral dosing

 F1   = 0
 F2   = 0
 F3   = 1

 TVBIO1 = THETA(1)
 AB     = 1 - TVBIO1
 AA     = LOG(TVBIO1/AB)
 BIO1   = EXP(AA+ETA(1))/(1 + EXP(AA + ETA(1)))
 BIO2   = 1 - BIO1

 TA1    = THETA(2)*EXP(ETA(2))
 MTT1   = THETA(3)*EXP(ETA(3))                ; COMP1 MEAN TRANSIT TIME
 N1     = THETA(4)*EXP(ETA(4))                ; COMP1 NO TRANSIT COMPS
 KTR1   = (N1+1)/MTT1                         ; COMP1 TRANSIT RATE CONSTANT

 LNFAC1 = LOG(2.5066) + (N1+0.5)*LOG(N1)-N1   ; COMP1 LN STERLING APPROXIMATION

 TA2    = THETA(5)*EXP(ETA(5))
 MTT2   = THETA(6)*EXP(ETA(6))                ; COMP2 MEAN TRANSIT TIME
 N2     = THETA(7)*EXP(ETA(7))                ; COMP2 NO TRANSIT COMPS
 KTR2   = (N2+1)/MTT2                         ; COMP2 TRANSIT RATE CONSTANT

 LNFAC2 = LOG(2.5066) + (N2+0.5)*LOG(N2)-N2   ; COMP2 LN STERLING APPROXIMATION


 V3   = (THETA(8)*(WT/70))*EXP(ETA(8)+BOVV)
 CL   = (THETA(9)*((WT/70)**0.75))*EXP(ETA(9)+BOVCLD)

 CLT4  = (THETA(10)*((WT/70)**0.75))*EXP(ETA(10))

 V4    = (THETA(11)*(WT/70))*EXP(ETA(11))          ; 30 FIX
 CL4H  = (THETA(12)*((WT/70)**0.75))*EXP(ETA(12))

 CLD   = CL - CLT4
```

```
 LN2   = LOG(2)
 K13   = LN2/TA1
 K23   = LN2/TA2
 K34   = CLT4/V3
 KE    = CLD/V3
 KE4   = CL4H/V4
 S3    = V3
 S4    = V4

$DES
 CD = A(3)/V3

 DADT(1) = EXP(LOG(BIO1*PODO1+0.00001)+LOG(KTR1)+N1*LOG(KTR1*T+0.00001)-KTR1*T-LNFAC1) -
K13*A(1)
 DADT(2) = EXP(LOG(BIO2*PODO2+0.00001)+LOG(KTR2)+N2*LOG(KTR2*T+0.00001)-KTR2*T-LNFAC2) -
K23*A(2)
 DADT(3) = K13*A(1) + K23*A(2) - K34*A(3) - KE*A(3)
 DADT(4) = K34*A(3) - KE4*A(4)


$ERROR (ONLY OBSERVATIONS)

 IPDICL = A(3)/V3
 IPD4OH = A(4)/V4

 IF(CMT.EQ.3)THEN
 IPRED = IPDICL
 ENDIF

 IF(CMT.EQ.4)THEN
 IPRED = IPD4OH
 ENDIF

 IRES  = DV-IPRED
 IF(STDY.EQ.0)THEN
 W3     = THETA(13)*IPRED
 ENDIF
 IF(STDY.EQ.1)THEN
 W3     = THETA(14)*IPRED
 W4     = THETA(15)*IPRED
 ENDIF

 IF(CMT.EQ.3)THEN
 W = W3
 ENDIF

 IF(CMT.EQ.4)THEN
 W = W4
 ENDIF

 IWRES = IRES/W

 Y = IPRED + W*EPS(1)


$THETA (0,0.7,1)    ; 1 BIO1
$THETA (0,0.1,1)    ; 2 TA1
$THETA (0,0.7,3)    ; 3 MTT1
$THETA (0,1,5)      ; 4 N1
$THETA (0,1,2)      ; 5 TA2
$THETA (0,1.3,3)    ; 6 MTT2
$THETA (0,41,100)   ; 7 N2
$THETA (0,5,50)     ; 8 V3
$THETA (0,50,100)   ; 9 CL
$THETA (0,100,500)  ; 10 CLT4
$THETA (30 FIX)     ; 11 V3
$THETA (0,20,200)   ; 12 CL4H
$THETA (0,0.21)     ; 13 Prop err adult
$THETA (0,0.18)     ; 14 Prop err child
$THETA (0,0.3)      ; 15 Prop err child 4OH
```

```
$OMEGA 1            ; 1 BIO1
$OMEGA 1.8          ; 2 TA1
$OMEGA 0.02         ; 3 MTT1
$OMEGA 0.6          ; 4 N1
$OMEGA 0.2          ; 5 TA2
$OMEGA 0.02         ; 6 MTT2
$OMEGA 2            ; 7 N2
$OMEGA BLOCK(2)
0.3
0.003  0.06         ; 8 V3 9 CL
$OMEGA 0.06         ; 10 CLT4
$OMEGA 0 FIX        ; 11 V4
$OMEGA 0.01         ; 12 CL4H
$OMEGA BLOCK(2)
0.9
0.002 0.04          ; 10 BOVV 11 BOVCL
$OMEGA BLOCK(2) SAME
;
;                   ; 12 BOVV 13 BOVCL

$SIGMA 1 FIX

$ESTIMATION PRINT=10 METHOD=1 INTER SIGDIG=5 NOABORT MAXEVAL=9999 POSTHOC MSFO=msfb201

$COVARIANCE

$TABLE ID TIME IPRED IWRES IRES                          NOPRINT ONEHEADER FILE=sdtab201
$TABLE ID AGE CMT WT HT CYP                              NOPRINT ONEHEADER FILE=cotab201
$TABLE ID AGE CMT SEX RACE CYP WT                        NOPRINT ONEHEADER FILE=catab201
$TABLE ID TIME AGE CMT WT CYP CLT4 CL4H IPRED            NOPRINT ONEHEADER FILE=patab201
```

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